CZECH ACADEMY OF AGRICULTURAL SCIENCES

Czech Journal of ANIMAL SCIENCE

ŽIVOČIŠNÁ VÝROBA

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

2

VOLUME 48
PRAGUE 2003
ISSN 1212-1819

CZECH JOURNAL OF ANIMAL SCIENCE

An international journal published under the auspices of the Czech Academy of Agricultural Sciences and financed by the Ministry of Agriculture of the Czech Republic

For information on Czech J. Anim. Sci. and full papers from Vol. 47 visit http://www.cazv.cz

Aim and scope: The journal publishes scientific papers and reviews dealing with the study of genetics and breeding, physiology, reproduction, nutrition and feeds, technology, ethology and economics of cattle, pig, sheep, goat, poultry, fish and other farm animal management.

The journal is cited in the bibliographical journal Current Contents – Agriculture, Biology and Environmental Sciences and abstracted in Animal Breeding Abstracts. Abstracts from the journal are comprised in the databases: Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Envi-ronmental Sciences, Czech Agricultural Bibliography, Food Science and Technology Abstracts, Toxline Plus.

Periodicity: The journal is published monthly (12 issues per year). Volume 48 appearing in 2003.

Acceptance of manuscripts: Two copies of manuscript should be addressed to: Ing. Zdeňka Radošová, Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic, tel.: +420 227 010 352, fax: +420 227 010 116, e-mail: edit@uzpi.cz.

Subscription information: Subscription orders can be entered only by calendar year (January–December) and should be sent to: Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic. Subscription price for 2003 is 1176 CZK or 214 USD.

 MK ČR E 4886

CZECH JOURNAL OF ANIMAL SCIENCE

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POKYNY PRO AUTORY

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Kaláb J. (1995): Changes in milk production during the sexual cycle. In: Hekel K. (ed.): Lactation in Cattle. Academic Press, London. 876–888.

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Relations between the course of parturition, body weights and measurements of Holstein-Friesian calves

Z. NOGALSKI

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ABSTRACT: The paper presents body weights and measurements of 217 Holstein-Friesian calves, and relations between the body measurements of calves and their mothers. The coefficients of phenotypic correlations between these measurements and the course of parturition were also determined. The frequency of assisted deliveries increased with increasing body weights and measurements of calves, and increasing ratio between the body measurements of calves and cows. The highest correlation was determined between the course of parturition and body weights of calves ($r = 0.27$), inner pelvic area in cows ($r = -0.51$), and the ratio between this area and body weights of calves ($r = -0.52$). The interrelation between the inner pelvic area and the course of parturition can be applied to a forecast of the quality of forthcoming delivery.

Keywords: dairy cows; parturition; dystocia; calves; body measurements; Rica pelvimeter

Difficult parturition, concerning mainly heifers (Brzozowski *et al.*, 1994; Tyczka *et al.*, 1996), results in increased perinatal calf mortality and postnatal cow mortality, lower fertility and productivity of a herd, and higher veterinary costs (Mangurkar *et al.*, 1984; Dobicki *et al.*, 1989; Daccarett *et al.*, 1993; Bortone *et al.*, 1994; Fourichon *et al.*, 2000). First of all, the course of parturition depends on the weight of a calf, degree of development of the genital tract, physical condition and hormonal profile of a cow, and fetus position (Johnson *et al.*, 1988; Naazie *et al.*, 1989). Disproportions in the body constitution of both cows and calves can also be a reason for difficult calving (Brzozowski *et al.*, 1998). It concerns especially the ratio between the pelvic area in a cow and the body weight of a calf (Johnson *et al.*, 1988).

The birth canal (uterus, uterine cervix, vagina, vestibule of the vagina and vulva), which can expand considerably, is limited by the pelvic girdle (Baier and Schaetz, 1972; Tyczka, 1998). The cow's pelvis is long, with visible fundus indentation (Baier and Schaetz, 1972). The pelvic axis is bent twice (Figure 1). During bearing down, the fetus must go through a rigid, almost inflexible narrowing. The most critical places are the pelvic inlet and outlet. The inlet, which has the form of an osseous, stretching-resistant ring, is characterized by transverse dimensions whereas the outlet – by longitudinal dimensions.

A positive correlation between the difficulty of calving and pelvic diameters was noted by Wollert *et al.* (1986) and Johnson *et al.* (1988). Naazie *et al.* (1989) observed no direct effect of the pelvic canal size on calving. According to Gaines *et al.* (1993), the ratio between the pelvis size and the body weight of a calf decides on the probability of difficult calving to the largest extent.

The investigations into the correlation between the course of parturition and body measurements in calves and cows were few and concerned Polish Black-and-White cattle (Brzozowski *et al.*, 1994), Polish Red-and-White cattle (Tyczka, 1998), triple cross cattle (Brzozowski *et al.*, 1998) and beef cross-breeds (Nogalski *et al.*, 2000).

The aim of the present research was to determine the relations between the course of parturition, body weights and measurements of Holstein-Friesian calves, paying special attention to the development of the pelvic skeleton in cows.

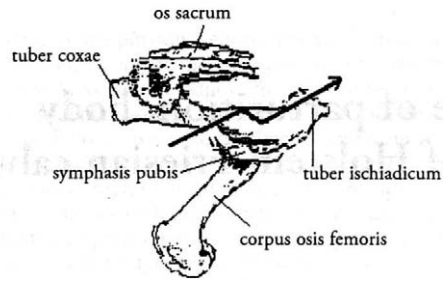

Figure 1. Route of foetus during parturition

MATERIAL AND METHODS

The studies were conducted for two years in three barns located in north-eastern Poland. 164 deliveries in Holstein-Friesian primiparas and 53 in cows were analyzed. Parturition took place in separate, long stalls where primiparas and cows were tied ca. two weeks before the expected date of calving. The course of parturition was evaluated according to the scale: 1 – normal, no assistance required; 2 – assistance of 1 person; 3 – difficult, assistance of several persons; 4 – very difficult, assistance of a veterinarian; 5 – cesarean section. Due to a low number of cases, groups 4 and 5 were treated as one. Newborn calves were weighed, and the following measurements were taken: a) height at withers, hips and pins; b) width of the head, at hips, pins and thurls; c) chest girth, fore cannon circumference, coronet circumference; d) length of the pelvis. The cows were measured between the 2nd and 3rd week after parturition (after edema regression). The external body measurements were taken, and the inner height and width of the pelvis were determined with Rica pelvimeter (Figure 2). The pelvis inner area is the product of the pelvis height and width (Patterson and Herring, 1997).

The acquired numerical data were subjected to an analysis of variance, using the following linear model:

$$Y_{ijklm} = \mu + A_i + B_j + C_k D_l + e_{ijklm}$$

where: Y_{ijklm} = value of the measurements taken
μ = mean for population
A_i = effect of ith course of parturition
B_j = effect of jth sex of the calf
C_k = effect of kth age of the cow at calving
D = effect of lth birth season
e_{ijklm} = random error

Significance of differences between the mean values was estimated using F test and Duncan's test. The coefficients of correlation between the course of parturition and body measurements of calves were also calculated.

RESULTS AND DISCUSSION

The body measurements of 217 calves (Tables 1 and 2) are presented according to the factors taken into account in the analysis of variance. Spontaneous calving or calving requiring the assistance of one person was recorded in 75.1% of cases. 37 deliveries required the assistance of two or three persons whereas 17 were complicated and the help of a veterinarian was indispensable. An increase in the fetus weight was accompanied by delivery complications. Other authors (Reklewska *et al.*, 1993; Naazie *et al.*, 1989; Wollert *et al.*, 1986) also reported that high fetus weight caused delivery complications, especially in heifers. According to Sakowski *et al.* (1989), the reason for delivery complications is too low body weight of a calf. The calves in whose case calving was classified as very difficult were characterized by the highest values of height, head width and chest girth. Their pelvises were the longest and the widest (measurement taken at hips). Except for the height at pins and pelvis length, these differences were confirmed statistically. Bull calves, compared with heifer calves, were statistically ($P \leq 0.05$) heavier, on average by 1.3 kg. Heifer calves were smaller than bull calves, but statistical differences were determined for the chest girth and the circumference of fore cannon only.

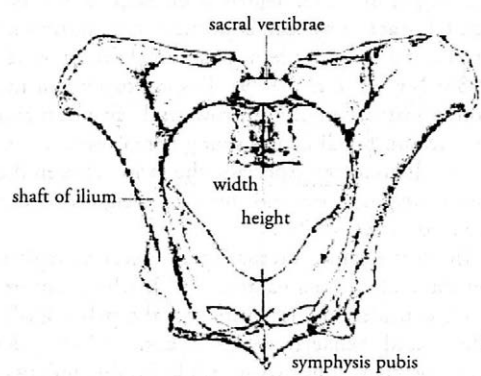

Figure 2. Inner dimensions of pelvis

Table 1. Body weights and measurements of calves according to the factors taken into account in the analysis of variance

Factor	n	Birth weight of calves (kg)		Height at withers		Height at hips		Height at buttock		Chest girth (cm)		Cannon circumference (cm)	
		$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v
Course of parturition													
easy calving	107	38.6^{Aab}	10.52	74.7^{A}	4.66	78.1^{b}	5.01	73.4	4.86	74.8^{A}	4.51	10.5	6.19
assistance of 1 person	56	40.0^{ac}	12.12	74.7^{B}	4.68	78.2^{a}	4.58	73.1	5.36	75.7	6.15	10.8	7.22
difficult	37	40.8^{b}	8.70	75.3^{a}	4.65	78.9	5.57	74.1	6.21	76.4	4.26	10.5	5.23
very difficult	17	42.2^{Ac}	7.85	77.1^{ABa}	4.02	80.5^{ab}	3.07	74.8	4.56	77.3^{A}	3.32	10.7	4.67
Sex of calves													
male	94	40.4^{a}	9.03	75.3	4.89	78.6	5.51	73.9	5.26	76.1^{a}	5.05	11.0^{A}	6.23
female	123	39.1^{a}	11.76	74.8	4.41	78.2	4.47	73.0	5.15	75.1^{a}	4.82	10.3^{A}	5.04
Age of cows													
primipara	164	39.4	9.98	74.7	4.87	78.3	5.08	73.2	5.30	75.4	4.91	10.6	6.41
pluripara	53	40.2	12.83	75.8	3.89	78.7	4.53	74.3	4.95	76.0	5.18	10.7	6.04
Calving season													
XI – IV	162	39.7	10.60	75.0	4.42	78.3	4.85	73.4	5.09	75.7	4.76	10.6	5.94
V – X	55	39.3	13.66	75.0	5.22	78.9	5.06	73.8	5.59	75.1	5.33	10.6	6.51
Total average	217	39.6	10.73	75.0	4.62	78.4	4.92	73.5	5.22	75.5	4.95	10.6	6.32

^{aA} means with the same subscripts differ significantly: small letters – $P \leq 0.05$; capital letters – $P \leq 0.01$

Table 2. Body measurements of calves according to the factors taken into account in the analysis of variance

Factor	n	Hoof circumference		Width of head		Width of hips		Width of trochanter		Width of buttock		Length of pelvis	
		$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v
Course of parturition													
easy calving	107	16.7^{a}	5.03	11.4^{a}	5.52	16.9^{a}	4.85	20.7	4.73	7.1	10.93	22.4	6.03
assistance of 1 person	56	17.1^{a}	5.11	11.2^{A}	6.31	17.1	5.15	20.8	9.32	7.2	12.51	22.5	5.62
difficult	37	16.9	5.95	11.5^{b}	6.40	17.3	5.61	20.8	4.75	7.3	14.22	22.4	6.07
very difficult	17	17.0	3.26	11.9^{Aab}	6.94	17.4^{a}	4.42	20.8	3.62	7.2	8.85	23.0	5.22
Sex of calves													
male	94	17.1	5.06	11.5	6.75	17.1	4.94	20.7	7.63	7.1	11.89	22.6	5.78
female	123	16.7	5.03	11.3	5.70	17.0	5.23	20.8	4.81	7.2	12.14	22.5	5.89
Age of cows													
primipara	164	16.8	4.94	11.3	6.36	17.0	4.94	20.7	6.23	7.1	11.90	22.5	5.64
pluripara	53	17.0	5.64	11.6	6.08	17.2	5.46	20.9	5.98	7.3	11.51	22.7	6.46
Calving season													
XI–IV	162	16.9	4.88	11.4	6.81	17.1	4.47	20.8	6.45	7.2	12.81	22.5	4.93
V–X	55	16.9	5.23	11.4	5.61	17.0	5.79	20.8	3.96	7.2	9.20	22.5	6.45
Total average	217	16.9	5.17	11.4	6.14	17.1	5.11	20.8	6.15	7.2	11.8	22.5	5.86

^{aA} means with the same subscripts differ significantly: small letters – $P \leq 0.05$; capital letters – $P \leq 0.01$

Table 3. Ratios (%) between the body measurements of calves and cows according to the factors taken into account in the analysis of variance

Factor	n	Body weight		Height at withers		Width of hips		Length of pelvis		Chest girth (cm)		Cannon circumference (cm)	
		$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v
Course of parturition													
easy calving	107	6.72^{ABC}	19.20	55.49	4.52	30.48^{ABC}	6.94	42.12	6.62	38.32^{ab}	7.83	56.38	6.89
assistance of 1 person	56	7.74^{A}	19.63	55.83	5.87	31.83^{A}	7.11	42.58	6.83	39.81^{a}	7.53	57.74	7.57
difficult	37	8.13^{B}	13.03	56.01	5.58	32.88^{B}	6.81	42.90	8.11	39.74^{b}	6.22	56.95	5.45
very difficult	17	8.18^{C}	13.69	56.78	5.59	32.74^{C}	6.57	43.45	7.35	39.19	4.69	57.45	6.56
Sex of calves													
male	94	7.62^{a}	19.94	55.95	5.54	31.57	7.74	42.81	6.59	39.37	7.17	59.08^{A}	6.78
female	123	7.13^{a}	18.79	55.63	4.87	31.29	7.44	42.22	7.32	38.74	7.75	55.26^{A}	5.27
Age of cows													
primipara	164	7.62^{A}	17.89	55.62	5.03	31.82^{A}	7.29	42.78^{A}	7.15	39.28^{a}	7.69	57.10	7.02
pluripara	53	6.48^{A}	20.83	56.22	5.35	30.15^{A}	6.97	41.54^{A}	6.12	38.19^{a}	7.10	56.35	6.53
Calving season													
XI–IV	162	7.32	19.17	55.66	4.83	31.54	7.74	42.45	7.07	39.16	7.69	56.97	6.61
V–X	55	7.38	20.54	56.10	6.21	31.05	7.42	42.56	6.80	38.60	6.84	56.74	7.58
Total average	217	7.34	19.62	55.77	5.18	31.41	7.58	42.48	7.06	39.01	7.56	56.91	6.87

^{aA} means with the same subscripts differ significantly: small letters – $P \leq 0.05$; capital letters – $P \leq 0.01$

Table 4. Coefficients of phenotypic correlation between the course of parturition and the body weights and measurements of calves, and the size of pelvic canals in cows

Specification	1	2	3	4	5	6	7	8	9	10	11	12	13
Course of parturition													
1. Birth weight of calves	0.27**	0.15*	0.15*	–	0.21**	–	–	–	0.20**	–	–	–0.51**	–0.52**
2. Height at withers		0.34**	0.34**	0.35**	0.71**	0.43**	0.31**	0.29**	0.43**	0.31**	0.30**	–	–0.67**
3. Height at hips			0.77**	0.65**	0.46**	0.36**	0.38**	0.39**	0.52**	0.48**	0.24**	–	–
4. Height at buttock				0.77**	0.45**	0.24**	0.35**	0.33**	0.42**	0.48**	0.26**	–	–0.16*
5. Chest girth					0.38**	0.22**	0.31**	0.37**	0.49**	0.52**	0.40**	–	–
6. Cannon circumference						0.44**	0.46**	0.29**	0.55**	0.41**	0.17*	–	–0.26**
7. Hoof circumference							0.53**	0.38**	0.36**	–	–	–	–0.24**
8. Width of head								0.24**	0.41**	0.37**	0.23**	–	–0.15*
9. Width of hips									0.41**	0.21**	0.23**	–	–
10. Width of buttock										0.52**	0.37**	–	–0.20**
11. Width of trochanter											0.45**	–	–
12. Pelvic area of cows												0.24**	–
13. Ratio: pelvic area of cows to birth weight of calves													0.70**

*$P \leq 0.05$; **$P \leq 0.01$

Calves delivered by heifers were lighter and characterized by insignificantly lower values of all body measurements. The season of calving had no significant influence on the body constitution of calves. Differences in fetus development, depending on the season, could be affected by heifer nutrition, pregnancy length and ambient temperature. In our own investigations calves born in the summer were lighter by 0.4 kg on average, whereas according to Holland and Odde (1992) this difference ranged from 0.5 kg to 2.7 kg.

Table 3 presents the ratios (%) between the body measurements of calves and their mothers. There was a significant correlation ($P \leq 0.01$) between the course of parturition and the ratio between their body weights. Higher body weight (%) of the fetus was connected with higher frequency of difficult calving. According to Holland and Odde (1992), in cattle the body weight of the fetus constitutes 5–10% (on average 7%) of the body weight of the mother. In our own studies this value was at an average level of 7.34%. The ratio (%) between the width of hips and chest girth in calves was also significantly affected by the course of parturition. In the case of spontaneous calving, or calving that required almost no assistance, the ratios between height at withers, width of hips, pelvis length and chest girth were lower.

As regards body weight and chest girth, the ratio between the measurements of bull calves and their mothers was significantly higher. Cow's age had a considerable effect on the ratios between body weights, widths of hips, pelvis lengths ($P \leq 0.01$) and chest girths ($P \leq 0.05$). Similarly like in the research carried out by Brzozowski *et al.* (1998), these ratios were higher in younger cows. The season of calving had no significant influence on differences in the ratios (%) between the body measurements of calves and cows.

Table 4 shows the coefficients of phenotypic correlation (r) between the course of parturition and body weights and measurements of calves, inner pelvic area in cows and the ratio between this area and body weights of calves. A highly significant correlation was observed between the body weights of calves and the course of parturition ($r = 0.27$). The highest correlation ($P \leq 0.01$) was determined for the course of parturition and the inner pelvic area ($r = -0.51$), and the ratio between this area and body weights of calves ($r = -0.52$). Naazie *et al.* (1989) reported $r = 0.46$ between the course of parturition in beef heifers and the ratio between the body weights of calves and pelvic area. An equally high correlation between the difficulty of calving and pelvic diameters was obtained by Wollert *et al.* (1986) and Johnson *et al.* (1988) whereas Naazie *et al.* (1989) observed no direct effect of the pelvic canal size on calving. The dependence between inner pelvic diameters and the course of parturition, stated in our own research, can be applied to a forecast of the quality of forthcoming delivery.

According to Gaines *et al.* (1993), the ratio between the pelvis size and body weight of a calf decides on the probability of difficult calving to the largest extent. Our own results indicate that a higher frequency of difficult deliveries in cattle is connected with higher body weight of the fetus and lower pelvic area. Figure 3 presents a correlation between the pelvic area in the cow and the body weight of the calf – the higher their quotient, the higher the probability of spontaneous calving.

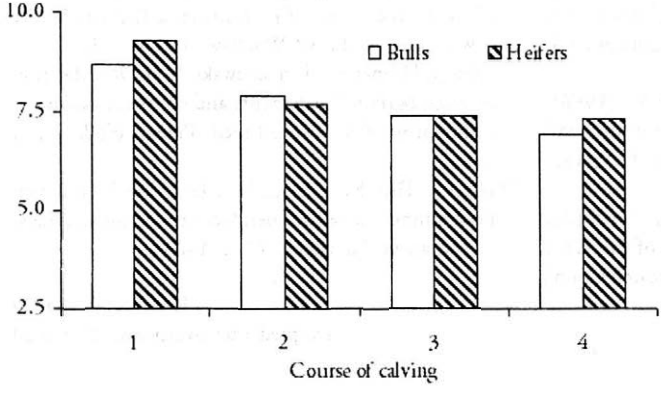

Figure 3. Correlation between the ratio of cow weight to calf birth weight and course of calving

The course of parturition was positively correlated with the height at withers and hips, and chest girth in calves. Brzozowski and Kaczmarek (1988) noted a correlation between the course of calving and chest width only.

CONCLUSIONS

The frequency of difficult calving in Holstein-Friesian cattle increased with increasing body weights and measurements of calves, and increasing ratio between the body measurements of calves and cows.

The highest correlation was observed between the course of parturition and body weights of calves, inner pelvic area in cows and the ratio between this area and body weights of calves.

The relation between inner pelvic diameters and the course of parturition, observed during the present investigations, can be applied to a forecast of the quality of forthcoming calving.

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Received: 02–05–20
Accepted after corrections: 03–01–20

ABSTRAKT

Závislosti mezi průběhem porodu, tělesnou hmotností a tělesnými rozměry telat holštýnsko-fríského plemene

V práci je uvedena tělesná hmotnost a tělesné rozměry 217 telat holštýnsko-fríského plemene, a dále poměr mezi tělesnými rozměry telat a jejich matek. Byly stanoveny koeficienty fenotypové korelace mezi těmito rozměry a průběhem porodu. Četnost asistovaných porodů se zvyšovala s vyšší tělesnou hmotností a tělesnými rozměry telat a s vyšším podílem mezi tělesnými rozměry telat a krav. Nejvyšší korelace byla zjištěna mezi průběhem porodu a tělesnou hmotností telat (r = 0,27), vnitřní plochou pánve u krav (r = –0,51) a poměrem mezi touto plochou a tělesnou hmotností telat (r = –0,52). Vzájemnou závislost mezi vnitřní plochou pánve a průběhem porodu lze využít k prognóze kvality nastávajícího porodu.

Klíčová slova: dojnice; porod; dystokie; telata; tělesné rozměry; pelvimetr Rica

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Journal of Forest Science	12	214
Veterinární medicína (Veterinary Medicine – Czech)	12	167
Czech Journal of Food Sciences	6	97
Plant Protection Science	4	64
Czech Journal of Genetics and Plant Breeding (Genetika a šlechtění)	4	64
Horticultural Science (Zahradnictví)	4	64
Research in Agricultural Engineering	4	64

Acid-base balance and blood gases in calves in relation to age and nutrition

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ABSTRACT: We conducted an extended study of acid-base balance indices and values of blood gases in six healthy calves from the 3rd to 24th week of age. The calves were kept in common rearing conditions. The study included the feeding of three types of diets – milk (milk replacer), transition to solid diet (milk replacer, hay, concentrates), and solid diet (hay, concentrates). Arterial blood was collected in the 3rd, 6th, 8th, 10th, 12th, 14th, 18th, and 24th weeks of age. The samples were analysed for blood pH, the partial pressure of carbon dioxide – pCO_2 (kPa), partial pressure of oxygen – pO_2 (kPa), actual bicarbonate – HCO_3^- (mmol/l), base excess – BE (mmol/l), saturation of haemoglobin with oxygen – O_2-sat (%), and alveolar-arterial gradient of oxygen – A-aDO_2 (kPa). Among the aforementioned indices, the most significant changes relating to age and nutrition were recorded for pO_2, O_2-sat, and A-aDO_2 (ANOVA – $P < 0.01$; $P < 0.001$). The average values of these indices were significantly higher after the 12th week of age compared with the earlier period. Significant effects of these factors on the blood pH and metabolic compartments of acid-base balance were recorded when, during transition and particularly during solid diet feeding, higher values were determined compared with the milk feeding period. The insignificant dynamics of average values, as well as time tendencies were recorded for pCO_2. The results showed that the milk-feeding period in calves was characterised by lower values of pH, HCO_3^-, BE, pO_2 and O_2-sat, and higher values of A-aDO_2, compared with the older calves.

Keywords: calves; acid-base balance; blood gases; arterial blood; milk feeding period; solid diet feeding period

The acid-base balance and blood gases play an important role in evaluating metabolism in calves. Due to environmental and body factors, there are frequent deviations with marked effects on the health state of animals. While a tendency to acidosis dominates, alkalosis is quite rare. The knowledge of physiology during the growth and development of calves can frequently help to clarify various pathological states occurring at this stage. The study of age effects on control mechanisms, functions of the viscera and body systems contributes to a better understanding of neonates, young, and developing animals (Bostedt and Schramel, 1982). This knowledge gives a better insight into young animals' diseases, particularly noninfectious diseases. It leads to more efficient therapy and prevention of diseases. Bouda and Jagoš (1984) reported that when diagnosing metabolic disorders in calves, there is a need to consider the developing immune system, labile homeostasis, functions and immaturity of body organs, and changes relating to the age of the animals. It was found by extensive studies of a large number of biochemical indices in calves of various age including their time-course changes and tendencies. These studies confirmed that body growth and development are accompanied by dynamic changes in many indices of haematological, protein, mineral, enzymatic, energy, and other profiles (Bouda *et al.*, 1980; Bostedt, 1983; Frerking *et al.*, 1983; Paulík *et al.*, 1983a,b; Bouda and Jagoš, 1984; Katunguka-Rwakishaya *et al.*, 1985; Paulík *et al.*, 1985; Slanina and Paulík, 1985; Hauser *et al.*, 1986; Dvořák *et al.*, 1986; Čupka, 1989). In calves, however, the relationships between acid-base balance, blood gases, age and growth have been investigated to a lesser extent. Moreover, a detailed study of acid-base balance requires not only venous blood analyses but also an examination of arterial blood, particularly in the assessment of blood gases (Hinchcliff and Byrne, 1991). The analyses of arterial blood have not been used in our conditions until now.

This paper studied the values of acid-base balance and blood gases in healthy calves kept in known conditions. Selected indices of arterial blood were analysed for half a year. The calves were reared in common housing and feeding technologies. Our aim was to record the age dynamics of acid-base balance and blood gases, and to characterise tendencies of their changes in calves fed various types of diet – milk (milk replacer), transitional (milk replacer and solid diet), and solid diet.

MATERIAL AND METHODS

Animals

An extended study of the dynamics of acid-base balance and blood gases was conducted in six healthy Slovak Pied calves (4 bulls and 2 heifers). The observation of the calves started at the age of 3 weeks, body weight 43–52 kg, one week after their adaptation to the clinic. The calves showed no health disorders during the whole experiment.

Feeding and housing

The calves were housed loosely in pens until the age of 8 weeks, then they were individually chained to a feeding trough. From their arrival at the clinic to the age of 4 weeks, the calves received only milk replacer at the dose of 2.5 l, three times daily (milk feeding period). The transition to a solid diet lasted from 4th to 10th week of age. During this period, the calves were fed gradually decreasing amounts of milk replacer (2.5–1.0 l), meadow hay, and concentrates (transitional feeding period). Between the 5th and 10th week of age, milk replacer was administered twice a day. The animals had free access to meadow hay, and the amount of concentrates was gradually increased from 0.1 up to 1.5 kg per calf and day. After the 10th week, the feeding of milk replacer was stopped and only a solid diet was fed (solid diet feeding period). During this period, the animals received meadow hay and concentrates, with free access to water.

Blood collection

Examination of acid-base balance and blood gases lasted from 3rd to 24th week of the calves' age. In sum, eight samplings were done in the 3rd (milk-feeding period), 6th, 8th, 10th (transitional feeding period), 12th, 14th, 18th, and 24th week (solid-diet feeding) of age. Blood collections were done at the same time of day, i. e. 4–5 hours after feeding in the morning. The arterial blood was collected by a direct puncture of the *arteria carotis communis* or *arteria axillaris* (Nagy *et al.*, 1998) into heparinized glass capillaries (Radiometer Copenhagen, type D551/20/150). After the collection, the samples were stored on ice and analysed by an ABL-4 analyser (Radiometer Copenhagen) within one hour of collection.

Analysed indices

In the analyses of acid-base balance and blood gases, the following indices were evaluated: blood pH, partial pressure of carbon dioxide – pCO_2 (kPa), partial pressure of oxygen – pO_2 (kPa), actual bicarbonate – HCO_3^- (mmol/l), base excess – BE (mmol/l), saturation of haemoglobin with oxygen – O_2-sat (%), and alveolar-arterial gradient of oxygen – A-aDO_2 (kPa). Values of pH, pCO_2, and pO_2 are measured directly by the analyser and are corrected to the temperature 39°C. The remaining indices are calculated by the analyser. The values of A-aDO_2 were calculated according to Donawick and Baue (1968).

Statistical analysis of results

The obtained results were processed by computer programme GraphPad InStat vers. 2.04 for means and standard deviations at different age of the calves' lives. The significance of age and nutrition effects on the studied indices was analysed by a one-way analysis of variance – ANOVA. In the case of any significance detected, Tukey-Kramer multiple comparison test was used to analyse significant differences between the mean values corresponding to the feeding periods. Differences between the first and subsequent samplings were checked by paired *t*-test.

RESULTS

Time-course changes in the analysed indices during the respective age periods are presented in

Table 1. The indices of acid-base balance and blood gas values in calves in relation to their age ($x \pm sd$)

Calves' age (weeks)	Parameter						
	pH	pCO_2 (kPa)	pO_2 (kPa)	HCO_3^- (mmol/l)	BE (mmol/l)	O_2-sat (%)	A-aDO_2 (kPa)
3	7.363 ± 0.013	6.1 ± 0.4	11.6 ± 0.7	25.8 ± 1.6	0.9 ± 1.4	95.0 ± 0.4	2.1 ± 0.7
6	7.400 ± 0.009^b	6.2 ± 0.5	10.9 ± 1.2	28.3 ± 2.0	3.6 ± 1.8	93.7 ± 2.4	2.7 ± 1.0
8	7.390 ± 0.001^a	6.4 ± 0.3	11.3 ± 0.8	28.4 ± 1.2	3.6 ± 1.0	95.4 ± 2.1	2.0 ± 0.5
10	7.388 ± 0.016	6.4 ± 0.2	12.7 ± 0.4	28.4 ± 1.6	3.5 ± 1.6	95.9 ± 0.6	0.7 ± 0.6
12	7.387 ± 0.014^a	6.1 ± 0.3	14.1 ± 0.7^b	27.4 ± 1.0	2.7 ± 0.9	96.9 ± 0.4^a	0.2 ± 0.4^c
14	7.392 ± 0.016	6.3 ± 0.3	13.7 ± 0.8^a	28.3 ± 1.5	3.5 ± 1.5	96.6 ± 0.6^a	0.2 ± 0.3^c
18	7.416 ± 0.011^a	6.6 ± 0.1	12.9 ± 1.5	31.1 ± 1.1^a	6.3 ± 1.1^a	96.2 ± 1.2^a	0.7 ± 1.0^a
24	7.406 ± 0.026	6.3 ± 0.4	13.8 ± 0.3^b	29.5 ± 3.2	4.5 ± 2.7	96.8 ± 0.3^a	0.1 ± 0.1^c
ANOVA	$P < 0.001$	n.s.	$P < 0.001$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.001$

n.s. = not significant

$^{a, b, c}$ = statistical significance of differences compared with the 3rd week of age (paired t-test, a = $P < 0.05$; b = $P < 0.01$; c = $P < 0.001$)

Table 1. During the period of observations, values of blood pH slightly increased compared with values recorded in the 3rd week of age. Significantly higher values were recorded in the 6th ($P < 0.01$), 8th, 12th, and 18th week ($P < 0.05$), respectively. The analysis of variance showed a significant age effect on the blood pH ($P < 0.001$). Throughout the period of observation, values of pCO_2 were almost the same with small differences. This indicates an insignificant age effect on pCO_2 values. Values of actual bicarbonate showed a tendency to increase with age, when significantly higher

Table 2. The acid-base balance and blood gases in calves during different feeding periods ($x \pm sd$)

Parameter	Feeding period			ANOVA
	milk	transitional	solid diet	
pH	7.363 ± 0.013^{bc}	7.393 ± 0.012^b	7.399 ± 0.020^c	$P < 0.01$
pCO_2 (kPa)	6.1 ± 0.4	6.3 ± 0.4	6.3 ± 0.3	n.s.
pO_2 (kPa)	11.6 ± 0.7^b	11.6 ± 1.2^c	13.7 ± 1.0^{bc}	$P < 0.001$
HCO_3^- (mmol/l)	25.8 ± 1.6^a	28.3 ± 1.6	29.0 ± 2.2^a	$P < 0.05$
BE (mmol/l)	0.9 ± 1.4^{ab}	3.5 ± 1.5^a	4.2 ± 2.1^b	$P < 0.01$
O_2-sat (%)	95.0 ± 0.8	94.9 ± 2.0^c	96.7 ± 0.7^c	$P < 0.001$
A-aDO_2 (kPa)	2.1 ± 0.7^c	1.9 ± 1.1^C	0.3 ± 0.6^{cC}	$P < 0.001$

n.s. = not significant

$^{a, b, c, C}$ in each row = values with similar superscripts differ significantly (a = $P < 0.05$; b = $P < 0.01$; c, C = $P < 0.001$)

values ($P < 0.05$) were recorded in the 18th week, compared with the first sampling (3rd week). The analysis of variance showed a significant age effect on HCO_3^- values ($P < 0.01$). Similarly, the values of BE showed a tendency to increase with significantly higher values in the 18th week ($P < 0.05$) with a significant age effect ($P < 0.01$). The time-course changes in pO_2 values were characterised by a significant increase with age ($P < 0.001$). Compared with the 3rd week of age, significantly higher values of pO_2 were recorded in the 12th, 24th ($P < 0.01$), and 14th ($P < 0.05$) weeks of age. A similar trend was also found for O_2-sat values with significantly higher values in the 12th, 14th, 18th, and 24th weeks of age ($P < 0.05$). An insignificant increase in A-aDO_2 values was observed in the 6th week of age. Except for this week, A-aDO_2 values had a tendency to decrease with significantly lower values in the 12th and 24th weeks of age ($P < 0.05$; $P < 0.001$) compared with the first sampling values. There were significant age effects on both O_2-sat ($P < 0.01$) and A-aDO_2 ($P < 0.001$) values.

The effects of nutrition on acid-base balance and blood gases are summarised in Table 2. The results showed that, except for the pCO_2 values, all the remaining indices were significantly influenced by the periods of nutrition (ANOVA – $P < 0.05$; $P < 0.01$; $P < 0.001$). The periods of transitional and solid diet feeding were characterised by insignificant differences between mean pH values. However, these values were significantly higher than the pH values recorded in the period of milk feeding ($P < 0.001$). The mean values of HCO_3^- significantly differed between the milk- and solid-diet feeding periods ($P < 0.05$). Compared with the milk-feeding period, significantly higher values of BE were recorded during the transitional ($P < 0.05$) and solid-diet ($P < 0.01$) feeding periods. Similar tendencies were observed in pO_2 and O_2-sat values with higher mean values during the period of solid diet feeding, compared with milk ($P < 0.01$) and transitional ($P < 0.001$) feeding periods. During the period of solid-diet feeding, the values of A-aDO_2 were significantly lower than those during the milk and transitional feeding periods ($P < 0.001$).

DISCUSSION

The mean blood pH values usually ranged within the limits reported for calves by Oakley *et al.* (1980) and Collie (1991). Higher values reaching even the range of alkalaemia – pH 7.45 and more – were reported by Vestweber *et al.* (1977) and Verhoeff *et al.* (1985). In relation to the age dynamics, our results of blood pH and metabolic compartments correspond to literary values to a certain extent. While slightly increasing trends of pH, HCO_3^-, and BE values were recorded in the present paper, Uhling and Gorznij (1993) reported insignificant changes in these indices from milk to solid diet feeding periods. On the other hand, a significant decrease in blood pH, HCO_3^-, and BE was observed by Reece (1980). Other authors (Reece and Wahlstrom, 1972; Reece and Hotchkiss, 1987), on the basis of a higher blood pH, assessed the state of acid-base balance as metabolic alkalosis with partial respiratory compensation. They suggested that the occurrence of metabolic alkalosis is associated with the mechanism of H^+ ions production in parietal abomasal cells and their secretion into the abomasal content. It is accompanied by production of bicarbonate ions absorbed into blood. This mechanism may apply in calves fed milk replacer or whole milk containing various concentrations of basic components. The type of diet can thus influence blood bicarbonate concentrations depending on the amount of secreted H^+ ions needed for reduction of abomasal pH to normal values. The time required to achieve abomasal pH optimal for digestion is longer when milk replacers are used. Vajda (1997) studied abomasal pH and acid-base balance in calves receiving normal and acidified milk. He found a decrease in pH below 4 as early as 2 hours after the feeding of acidified milk while this process lasted up to 5 hours when normal milk was fed. These differences were associated with significant differences in blood pH, HCO_3^-, and BE with lower values in animals fed acidified milk. A tendency towards a decrease in blood pH and bicarbonates (acidogenic effect) was observed by Lebeda (1969) and Lebeda and Buš (1967) in calves fed a semisynthetic diet – milk replacer composed of skimmed milk – compared with calves fed whole milk. In our work, feeding the milk replacer had a similar acidifying effect reflected in blood pH and bicarbonate values. A frequent shift in acid-base balance towards acidaemia was also reported by Bouda and Jagoš (1984) in calves during the transition from natural milk to milk replacer or solid diet feeding. In the evaluation of possible effects on blood pH in young and older calves, Steinhardt *et al.* (1995) emphasised the importance of lactate levels. The authors observed increased lactate lev-

els in young calves and excluded a possibility that this increase was due to increased muscle exertion before blood sampling. These findings, confirmed also by Thielscher (1994), indicate an insufficient supply of oxygen to the body and tissue hypoxia with consequently increased anaerobic glycolysis. It is associated with increased concentrations of H^+ ions and decreased blood pH. As late as at 90 days of age or more, the respiratory capacity becomes sufficient for oxygen supply, which is reflected (also in our study) in the increase in pO_2 and O_2-sat values. The reason for the insufficient oxygen supply in calves should also be sought in insufficient transport capacity for oxygen due to anaemia (low packed cell volume and haemoglobin levels), which is also manifested by a higher pulse frequency in calves fed milk diet (Steinhardt *et al.*, 1994).

The pCO_2 values of arterial blood determined in our study are comparable with the values reported by Reece and Wahlstrom (1972), Oakley *et al.* (1980), and Reinhold and Födisch (1993). Markedly reduced mean pCO_2 values in the arterial blood of calves (5 kPa and less) were recorded by Vestweber *et al.* (1977) and Collie (1991). Our results of pO_2 ranged around 11 kPa during the milk and transitional feeding periods, and around 13 and more kPa during the period of solid diet feeding. These values are similar to the values reported in healthy calves of similar age (Luitjens, 1990; Klein, 1996). Slightly higher values of pO_2 were observed by Oakley *et al.* (1980) and Verhoeff *et al.* (1985), who found average values 13.9 to 14.2 kPa and individual values over 15 kPa. The evaluation of O_2-sat and A-aDO_2 is limited because of the lack of available published data. Therefore, we suggest that our results contribute to the current knowledge. Ninety-five percentage haemoglobin saturation with oxygen is assumed to be optimal. However, our results obtained in the 6th week of age as well as the results of other authors (Uhling and Gorznij, 1993; Klein, 1996) indicate a possibility of lower values also in healthy calves, especially in younger categories. Our result of A-aDO_2 corresponds to the A-aDO_2 values reported in healthy calves by Donawick and Baue (1968), Uhling and Gorznij (1993), and Klein (1996). Uhling and Gorznij (1993) found no significant correlation between the pO_2 and age, a tendency of O_2-sat values to increase without significant differences between age groups, likewise of A-aDO_2 values that showed larger variations. These authors suggested that there is no need to consider age effects when evaluating the results of pO_2 analysis. However, our results indicate that it is important as, for example, the difference between the 3rd and 24th week of age exceeded 2 kPa. These results correspond to the results of Lekeux *et al.* (1984) and Gustin *et al.* (1988).

Changes in lung functions, blood gases as well as A-aDO_2 values with age, body growth or organ development are related to morphological lung changes in growing calves. Morphological studies of such changes are based on the fact that the viscera have heterogeneous growth and development, both in pre- and post-natal life periods, which is also associated with functional changes. In these periods of proportional changes, the body requires not only an intake of nutrients but also an increased oxygen supply for oxidative processes. Although young animals have relatively higher lung weight, they have not relatively larger lung volume because their lungs consist of more undifferentiated tissue and are less airy (Hörnicke, 1969). The lung increase during body growth is accompanied by the growth of a new alveolar system resulting in some regulation of air exchange. It was also confirmed by Přibyl (1980), who studied changes in the lung tissue of 114–166 day-old calves and found extreme changes characterised by the enlargement of lung alveoli and the thinning of lung interstitial tissue. The highest proportional decrease in the lung interstitial tissue, as a mirror image of increase in the percentual proportion of alveoli, was recorded between the 3rd and 6th month of age. Moreover, Přibyl (1980) found larger alveoli in heifers than in bulls. When characterising the main features of post-natal lung growth in calves from birth to 150 days, Castleman and Lay (1990) found that new-born calves have fully developed alveoli. The relationship to age, or growth is characterised by an increase in the total surface of alveoli and their number, more marked in calves over 30 days. Besides the increasing lung volume and weight, there is also an increase in bronchiolus diameter. Following these findings, the authors concluded that post-natal development of other alveoli results in enlarging the proportion of tissues enabling the exchange of gases. The aforementioned data indicate that tendencies of pO_2, O_2-sat, and A-aDO_2 values recorded in our study reflect morphological changes in the lung parenchyma. Contrary to pO_2 values, we did not observe any marked changes in pCO_2 values during the growth of animals. It resulted from different ability of blood gases to

diffuse through the alveolar-capillary membrane, which is approximately 20 times higher in CO_2. The release of CO_2 thus seems to be sufficient to avoid hypercapnia and respiratory acidosis, respectively, even in less mature lungs. It corresponds to the findings of other authors who found insignificant changes in pCO_2 values throughout a similar age period (Lekeux *et al.*, 1984; Collie, 1992). A long study of lung functions in cattle showed pCO_2 values to be lower than in young animals as late as in animals over one year (Lekeux *et al.*, 1984).

With different rearing systems and requirements for growth intensity, the growing body is withstanding serious problems. Moreover, it is just the youngest animals that have a peculiar labile ion balance (Lebeda and Buš, 1967; Hartmann *et al.*, 1984). In our study, despite of various time-course changes in the analysed indices, we consider the normal functions of compensatory mechanisms in controlling possible deviations in respiratory or metabolic compartments of acid-base balance. It was indicated by pH values within the normal range (except 3rd week) as pH is the principal parameter indicating balanced or disturbed acid-base state of the body.

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Received: 02–05–13
Accepted after corrections: 03–01–07

ABSTRAKT

Acidobázická rovnováha a krvné plyny u teliat vo vzťahu k veku a výžive

Vykonali sme dlhodobé sledovanie parametrov acidobázickej rovnováhy (ABR) a krvných plynov (KP) u šiestich klinicky zdravých teliat v období od 3. do 24. týždňa veku za podmienok odchovu uplatňovaných v mnohých našich chovoch. Uvedený časový úsek zahŕňal tri obdobia výživy – mliečne (mliečna kŕmna zmes), prechod na rastlinné (mliečna kŕmna zmes, seno, jadrové krmivo) a výlučne rastlinné kŕmenie (seno, jadrové krmivo). Odber arteriálnej krvi bol vykonaný v 3., 6., 8., 10., 12., 14., 18. a 24. týždni veku. Hodnotili sme aktuálne pH krvi, parciálny tlak oxidu uhličitého – pCO_2 (kPa), parciálny tlak kyslíka – pO_2 (kPa), aktuálny bikarbonát – HCO_3^- (mmol/l), prebytok báz – BE (mmol/l), saturáciu hemoglobínu kyslíkom – O_2-sat (%) a alveolárno-arteriálny gradient kyslíka – A-aDO_2 (kPa). Z uvedených parametrov najvýznamnejšie zmeny hodnôt vo vzťahu k veku a obdobiu výživy teliat

boli zistené u pO_2, O_2-sat a A-aDO_2 (ANOVA – $P < 0,01$; $P < 0,001$). Prejavilo sa to významne vyššími priemernými hodnotami týchto parametrov v období nad 12 týždňov veku v porovnaní s predchádzajúcim obdobím. Významný vplyv uvedených faktorov bol zaznamenaný aj na hodnoty pH a metabolických ukazovateľov acidobázickej rovnováhy krvi, kde priemerné hodnoty v období prechodnej, ale hlavne rastlinnej výživy boli vyššie ako v období výživy mliečnej. Nevýznamná dynamika v priemerných hodnotách ako aj nevýznamné trendy dynamiky vo vzťahu k veku boli zistené u pCO_2. Výsledky poukázali na to, že obdobie výlučne mliečnej výživy teliat je v porovnaní so staršími teľatami charakterizované nižšími hodnotami pH, HCO_3^-, BE, pO_2, O_2-sat a vyššími hodnotami A-aDO_2. Výsledky dosiahnuté pri O_2-sat a A-aDO_2 sú doplnením doterajších poznatkov a hodnôt, pri ktorých je v literárnych zdrojoch menej údajov. Zohľadnenie naznačených trendov zmien sledovaných parametrov vo vzťahu k veku a výžive možno uplatniť pri hodnotení ABR a KP u rôznych vekových kategórií teliat v rámci klinicko-laboratórnej diagnostiky porúch zdravotného stavu.

Kľúčové slová: teľatá; acidobázická rovnováha; krvné plyny; arteriálna krv; mliečna výživa; rastlinná výživa

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Investigation of polymorphisms in the porcine myostatin (*GDF8*; *MSTN*) gene

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ABSTRACT: Myostatin is a growth factor controlling proliferation of myoblasts in embryonic development. Mutations in coding sequences of bovine myostatin (*GDF8*) gene lead to muscle hyperplasia suggesting its inhibitory function on myoblast proliferation. The present study was designed to scan for mutations in porcine *GDF8* promoter (397 bp), exon 2 with flanking regions (631 bp) and exon 3 with flanking regions (899 bp) using PCR-RFLP, SSCP and DSCP analyses. *Mnl*I and *Dra*I enzymes were used to determine restriction polymorphism in the promoter region of *GDF8*. In *Dra*I site 3 pigs were of *AT* and 291 of *TT* genotype. No restriction polymorphism was detected using *Mnl*I enzyme. SSCP analysis in 12% polyacrylamide gel did not reveal any differences in the migration rate between PCR products. SSCP and DSCP analyses of respectively 30 and 14 PCR products encompassing exon 2 did not reveal any mutations either. In total 314 pigs were analysed for *Taq*I polymorphism in exon 3; out of them 13 were *TT* homozygotes, 112 were *CT* heterozygotes and 189 were *CC* homozygotes. No SSCP or DSCP polymorphism was detected in this region. Generally the porcine myostatin gene seems to be more conserved than in cattle. Additional statistical comparison of some carcass traits between *CC* and *CT* animals was performed that did not show any significant differences.

Keywords: myostatin gene; *GDF8*; pig; mutations; polymorphism

Myostatin is a growth factor of the TGF-β superfamily. TGF-β growth factors play a role in the regulation of embryonic development and tissue homeostasis in adults (Sonstegard *et al.*, 1998). *In vitro* they are known to block myogenesis, hematogenesis and enhance chondrogenesis as well as epithelial cell differentiation. The myostatin gene (*GDF8*) is expressed in skeletal muscle (Arnold and Winter, 1998) both at prenatal and postnatal stages of pig and cattle development (McPherron *et al.*, 1997; Ji *et al.*, 1998). Apart from skeletal muscle *GDF8* was found to be expressed postnatally in adipose tissue of cattle and mice (McPherron *et al.*, 1997; Ji *et al.*, 1998) and mammary gland of pigs (Ferrel *et al.*, 1999). Since the myostatin protein has been found in blood plasma, it is highly possible that myostatin receptors are localised in various tissues (Gonzalez-Cadavid *et al.*, 1998). In the extracellular environment myostatin protein forms dimers that cleave into biologically active forms (Grobet *et al.*, 1997; Zhu *et al.*, 2000). The molecular structure of myostatin protein is conserved across vertebrates so that its C-terminal sequences are 100% identical in mice, rats, humans, pigs and chickens (McPherron and Lee, 1998). In muscle development myostatin prevents excessive proliferation of myoblasts (Bass *et al.*, 1999). Therefore mutations disrupting the function of this protein result in the formation of supernumerary muscle fibres, a phenomenon called muscle hyperplasia (McPherron *et al.*, 1997; Grobet *et al.*, 1998). It is common in Belgian Blue, Piemontese and Charolais cattle breeds, where a 20–25% increase in muscle mass can be observed. Interestingly, this phenomenon is accompanied by a reduction of intramuscular fat, connective tissue and some internal organs. Induced mutation of murine *GDF8* gene, leading to disruption of its protein structure and subsequent loss of biological activity, resulted in a 200–300% increase in the mass of individual skeletal muscles (McPherron and Lee, 1998). Interestingly, muscle hyperplasia in mice

was accompanied by hypertrophy (increased diameter of muscle fibres) while in cattle only muscle hyperplasia was observed (Kambadur *et al.*, 1997; McPherron *et al.*, 1997; Szabó *et al.*, 1998). Zhu *et al.* (2000) suggested that the experimentally diminished expression of *GDF8* resulted in muscle hypertrophy while the increased expression resulted in muscle hyperplasia.

Heavy muscling in pigs was found to be a consequence of muscle hypertrophy, especially remarkable in Pietrain and Belgian Landrace. However, muscle hyperplasia in PIC pig commercial line was also described recently (Klosowska *et al.*, 1998). The muscle composed of a higher number of muscle fibres per unit area possesses more favourable technological properties such as tenderness. Moreover, a higher number of muscle fibres (hyperplasia) accounts for better and more efficient growth of lean muscle mass (Christensen *et al.*, 2000). Therefore it would be of interest to find mutations in the porcine myostatin (*GDF8*) gene that would potentially account for heavy muscling and good meat quality.

MATERIAL AND METHODS

Pigs from two farms were used in this study

Farm I. 30 Pietrain pigs (Pi); 30 Polish Landrace pigs (PL^{I}); 30 Zlotnicka Pied pigs (ZP); 30 crosses of Pietrain × Zlotnicka Pied sows mated to Pietrain boars [Pi × (Pi × ZP)]; 39 pigs of Stamboek commercial line; 115 crosses of Large White × Polish Landrace sows mated to Pietrain boars ($TORHYB^{I}$); 39 pigs of $TORHYB^{II}$ (id., additional selection in maternal line for increased muscularity); 20 Polish Landrace sows bred to Dutch and Danish Landrace boars (PL^{II}).

Farm II. 59 Large White × Pietrain crosses (LW × Pi); 32 Large White × Pulawska crosses (LW × Pul); 11 Large White pigs (LW); 32 Pietrain (Pi) and 12 PIC commercial line pigs.

Blood was collected into 10 ml tubes with EDTA*K_2 and stored at –20°C or –70°C (longer storage periods). 100 µl of blood was subsequently used for DNA isolation. The method of isolation was based on that of Kawasaki (1990) and Coppieters *et al.* (1992).

Primer pairs (Table 1) were designed using the Primer3 Website (www-genome.wi.mit.edu/cgi-bin/primer3_www.results.cgi v 0.2). GenBank databases were used to design primer pairs for the following myostatin fragments:

a) *GDF8 promoter* – 397 bp long fragment of *GDF8* promoter region (GenBank accession number AJ133580).

b) *GDF8 exon2* – 631 bp fragment encompassing part of intron 1, exon 2 and part of intron 2 of the *GDF8* gene (GenBank accession numbers AJ237662 and AJ237920).

c) *GDF8 exon3* – 899 bp fragment encompassing part of intron 2, exon 3 and noncoding part of the 3' end of *GDF8* gene (GenBank accession numbers AJ237920 and AF033855).

PCR reactions were performed in 10 µl final volume, containing 4 pM of each primer (INTERACTIVA Biotechnologie GmbH, Ulm, Germany), 800 µM dNTPs (PE Biosystems, New Jersey, U.S.A), 1.5 mM $Mg(oAc)_2$ and 0.3 U HotStar*Taq*™ Polymerase (QIAGEN, Hilden, Germany) for amplification of *GDF8 exon2* and *exon3* or DyNAzyme™ II DNA Polymerase, (Finnzymes, Espoo, Finland) for amplification of *GDF8 promoter* in respective buffers. Approximately 0.1 µg of DNA was added to each reaction mixture. Amplification conditions are shown in Table 2.

Table 1. Primer pairs designed to amplify parts of the porcine myostatin gene

Part of *GDF8* gene	Primer sequence
GDF8-promoter	Forward: 5' TTT TTG AGG AAA AAG ACA TTT CAA 3' Reverse: 5' ACA ACT TGC CAC ACC AGT GA 3'
GDF8-exon2	Forward: 5' TTT CAT CCA CTC TTC ATT CCT TTA CAG 3' Reverse: 5' GTT ATT TTC CAC TAC TAC TCA TTC ACA 3'
GDF8-exon3	Forward: 5' CTG CCT CTC TCT CTC TTC TCT GTC CTC 3' Reverse: 5' CTT TTT ATT GTA TGA TTT GTT TTG ATG 3'

Analysis of restriction polymorphisms

5 µl of *GDF8 promoter* PCR product was digested with 2U of *Dra*I or *Mnl*I restriction enzyme overnight, followed by 3% or 4% agarose gel electrophoresis, respectively. 5 µl of the *GDF8 exon3* PCR product was digested overnight with 4-6U of *Taq*I restriction enzyme and subsequently analysed in 2.5% agarose.

Analysis of single strand (SSCP) and double strand (DSCP) conformation polymorphisms:

SSCP and DSCP analyses were performed to search for mutations in exon 2 of the *GDF8* gene. Prior to this analysis PCR products were digested with 5U of *Dde*I restriction enzyme to obtain shorter fragments more suitable for SSCP analyses. Restriction enzymes were selected by an analysis by DNASIS1 (Hitachi Software).

Various combinations of polyacrylamide gels (5, 8, 15% concentration, with or without addition of 5% glycerol) were used to optimize the efficiency of mutation detection.

Statistics

Carcass characteristics of pigs showing different myostatin genotypes in exon 3 were compared using Least Square Means Test, general linear models procedure, SAS Institute Inc., Cary, NC, USA:

$$y_{pmnor} = \mu + GDF8_p + \text{Breed}_m + RYR1_n + \text{Sex}_o + \beta(SW_{pmnor} - SW) + e_{pmnor}$$

where: μ = mean value in the population

$GDF8_p$ = effect of myostatin genotype (described as *CC, CT, TT*)

Breed_m = breed effect

$RYR1_n$ = effect of stress susceptibility genotype

Sex_o = effect of sex (female or castrate)

$\beta(PSW_{pmnor} - PSW)$ = linear regression of y on pre-slaughter weight

e_{pmnor} = random effect

The following carcass traits were compared: half-carcass lean meat, fat thickness over shoulder, fat thickness over the last rib, fat thickness at *sacrum* I, at *sacrum* II and at *sacrum* III, mean backfat thickness, loin (total mass, meat, bone and skin + subcutaneous fat), loin 'eye' area, tenderloin, ham (total mass, meat, bone and skin + subcutaneous fat).

As the dissection method of carcasses of animals from Farm I (Pietrain, Polish Landrace[I], Zlotnicka Pied and crosses of Pietrain × Zlotnicka Pied sows mated to Pietrain boars) was different from the dissection method used for animals from Farm II (Stamboek, TORHYB[II] and Polish Landrace[II]), statistical comparisons were done separately for both groups.

RESULTS

GDF8 promoter

PCR-RFLP analysis was performed using two restriction enzymes. Names of the alleles are due to mutations occurring as transversion A→T (*Dra*I recognition site) or transition C→T (*Mnl*I recognition site):

with *Dra*I restriction enzyme:

allele *A* (259 + 65 + 60 + 13 bp bands)

allele *T* (324 + 60 + 13 bp bands)

with *Mnl*I restriction enzyme:

allele *C* (186 + 117 + 94 bp bands)

allele *T* (280 + 117 bp bands)

Table 2. PCR amplification conditions

Part of *GDF8* gene	Denaturation in Ist cycle	Denaturation		Annealing		Elongation		Final extension	Number of cycles
	°C/s	°C	s	°C	s	°C	s	°C/s	*n*
GDF8-promoter	95/120	94	45	57	45	72	60	72/600	35
GDF8-exon2	95/900*	94	30	58	60	72	60	72/300	35
GDF8-exon3	95/900*	94	60	55	60	72	90	72/420	36

*Extended time required for activation of HotStar*Taq*™ polymerase (according to manufacturer's directions)

Table 3. Allele frequencies in *GDF8* promoter and exon 3 polymorphic sites in different pig breeds and crosses (*n* = number of tested animals)

Farm	Breed/cross	Promoter/*DraI*			Promoter/*MnlI*			Exon 3/*TaqI*		
		n	*A*	*T*	*n*	*C*	*T*	*n*	*C*	*T*
I	Pietrain	29	0	1.00	13	0	1.00	24	0.58	0.42
	Polish Landrace[I]	46	0	1.00	15	0	1.00	28	0.78	0.22
	Zlotnicka Pied	23	0	1.00	14	0	1.00	28	0.78	0.22
	Pi × (Pi × ZP)	–	–	–	–	–	–	30	1.00	0
	TORHYB[I]	84	0.02	0.98	–	–	–	103	0.77	0.23
	Stamboek	32	0	1.00	15	0	1.00	34	0.90	0.10
	TORHYB[II]	29	0	1.00	15	0	1.00	36	0.85	0.15
	Polish Landrace [II]	–	–	–	–	–	–	19	0.66	0.34
II	LW × Pi	17	0.03	0.97	12	0	1.00	–	–	–
	Large White	11	0	1.00	–	–	–	–	–	–
	LW × Pul	23	0	1.00	–	–	–	–	–	–
	Pietrain[K]	–	–	–	–	–	–	–	–	–
	PIC	–	–	–	–	–	–	12	0.33	0.67

The *DraI* polymorphism was first described by Stratil and Kopečny (1999). A majority of pig breeds tested by the authors were homozygous for *T* allele (Czech Meat Pig, Black Pied Prestice, Pietrain, Hampshire and Duroc). In other breeds, such as LW, Landrace and Meishan the *A* allele occurred, however at very low frequencies (0.05–0.21). Results of the present study are in concordance with the above-mentioned paper. Totally 294 randomly selected animals of eight breeds or crosses were analysed for *DraI* polymorphism in the myostatin promoter region. Except for one heterozygous individual of LW × Pi and two of TORHYB[I] line all remaining animals were *TT* homozygotes (Table 3).

The *MnlI* restriction enzyme was selected for the present study due to analysis of the AJ133580 database. All of the 84 randomly selected pigs (Pietrain, Polish Landrace[I] and Polish Landrace[II], Zlotnicka Pied, Stamboek, TORHYB[II], LW × Pi) were *TT* homozygotes. Therefore no further analysis of this mutation was carried out.

GDF8 exon2

This part of *GDF8* gene was analysed solely by SSCP and DSCP methods. As the chance of mutation detection decreases with an increase in the length of PCR product (Cotton, 1999), we used *DdeI* restriction enzyme to cut the 631 bp long PCR product into 320 + 165 + 146 bp fragments. The analysis was performed on 30 randomly selected pigs of 9 breeds or crosses including Pi, ZP, PL[I], [Pi × (Pi × ZP)] and Stamboek from Farm I and LW × Pul, Pi, LW, LW × Pi from Farm II. Different combinations of polyacrylamide gels were used (5% with glycerol, 8% with or without glycerol). PCR products of one individual were analysed in two different combinations at least. Moreover, PCR products of 14 pigs were analysed by DSCP in 15% polyacrylamide gel. DSCP method is similar to SSCP with the exception that whole, nondenatured DNA is electrophoresed. The 15% gel concentration was used according to Cotton (1999), who suggested 15–20% polyacrylamide gels as optimal. However, no differences in the migration rate were observed in the present study.

GDF8 exon3

The *TaqI* restriction site in exon 3 of *GDF8* gene was discovered by Stratil and Kopečny (1999). The PCR product used in the present study possessed one variable and one stable restriction site for this enzyme, resulting in the following alleles:

allele *C*: 493 + 378 + 28 bp bands
allele *T*: 493 + 406 bp bands.

302 pigs of Farm I and 12 of Farm II were analysed for this polymorphism revealing a high frequency of *C* allele (Table 3). *TT* homozygotes were found only in PL^{II} (6 pigs/31.6%) and PIC (6 pigs/50%) and one $TORHYB^{I}$ (0.9%).

For this polymorphism statistical comparison of some carcass traits between *CC* (in total 189 animals) and *CT* (in total 112 animals) genotypes was performed. In the pigs of Farm II, where 13 *TT* homozygotes were found, all three possible genotypes were included in statistics. However, standard deviations for the traits of *TT* homozygotes were remarkably high due to a low number of animals carrying this genotype. No differences in carcass traits were found between the genotypes indicating that this mutation has no effect on the myostatin protein function in myogenesis.

DISCUSSION

The coding sequence of myostatin gene encompasses 1 128 bp (McPherron and Lee, 1998) and two transcripts of this gene were found in porcine muscle: 800 and 1 500 bp long (Sonstegard *et al.*, 1998). The porcine gene is composed of three exons with lengths of 373, 374 and 381 bp, respectively. The active form of protein comprises 376 amino acids (Stratil and Kopečny, 1999). The promoter region of human *GDF8* gene contains E-box sequence that binds muscle regulatory factors of MyoD family. It is supposed that myostatin is a downstream target of MyoD proteins (Ferrel *et al.*, 1999).

Nine mutations of *GDF8* are known in cattle, 5 of them located in coding sequences completely disrupt the protein's function and lead to muscle hyperplasia (Grobet *et al.*, 1998). Differences in the expression levels of both myostatin and MyoD factors in double muscled cattle compared to normal cattle were reported. The interactions of myostatin with different sets of genes that were fixed during selection across various breeds of double muscled cattle can account for differences in the observed level of muscle hyperplasia (Bass *et al.*, 1999). Similar interactions can account for the fact that myostatin inactivation in mice results both in muscle hypertrophy and hyperplasia while in cattle only in hyperplasia (McPherron *et al.*, 1997; Szabó *et al.*, 1998). Additionally, the composition of muscle fibres in an individual muscle plays a role since higher levels of *GDF8* expression were found in white than in red muscle of pigs (Ji *et al.*, 1998). For that reason the efforts to select heavy muscled animals should not focus on one gene only and the influence of genetic background needs to be considered as well.

Porcine myostatin is an object of growing interest. Some mutations in porcine *GDF8* gene were found, however their occurrence was very low (Stratil and Kopečny, 1999 and present study) indicating that myostatin protein is more stable in this species than in cattle. The presented results confirmed previous observations that porcine *GDF8* is much more conserved and stable than bovine. However the genotype frequency in *GDF8 exon3* determined by *TaqI* restriction enzyme can be confusing. It is interesting that the occurrence of *TT* homozygotes was very low while the heterozygous genotype was relatively frequent. It is also remarkable that the highest frequency of *TT* homozygotes was characteristic only of PIC line where the muscle fibre number per unit area was 29% higher if compared with LW × PL crosses due to muscle hyperplasia (Klosowska *et al.*, 1998). In the present study *TaqI* genotype was determined only in 12 PIC animals so that further investigation is necessary to explain if the higher frequency of *T* allele can account for the increased number of muscle fibres in this line of pigs. However, the statistical analysis did not reveal any differences in loin and ham meatiness nor in half-carcasses between the pigs of those three genotypes. Although the $C \rightarrow T$ mutation does not change a type of encoded amino acid (Stratil and Kopečny, 1999), it can influence mRNA stability. Jiang and Gibson (1999) suggested a similar effect of silent mutation in leptin gene. The fluctuations of *GDF8* expression level during development are necessary for proper regulation of *MyoD* genes and thus for precise regulation of the timing of myoblast proliferation and differentiation. Piglets of low birth weights were found to express lower levels of myostatin than their heavier littermates. Their muscle also contained less fibres (Ji *et al.*, 1998). It is not known whether myostatin influences only muscle formation or has a function in the regulation of muscle metabolism (Ji *et al.*, 1998). In HIV infected men myostatin was found to be involved in muscle wasting (Gonzalez-Cadavid *et al.*, 1998).

Unlike cattle, heavy muscling in pigs results rather from muscle hypertrophy than from hyperplasia indicating that the myostatin function

remains rather undisturbed. Together with the results of Stratil and Kopečny (1999), the present study provides evidence that myostatin is a conserved and stable protein in pigs. However other mutations can exist, including non-coding regions of myostatin gene with an effect on its function in pigs. Previous investigations of Marco *et al.* (1999) into mutations in *GDF8* gene in double muscled Texel sheep did not reveal any changes within coding sequences either. However linkage analyses indicated the presence of some markers for muscle characteristics in non-coding sequences of ovine *GDF8* gene (Marco *et al.*, 1999). The molecular basis of heavy muscling may be more complicated considering that muscle development is a complex result of the activity of numerous factors so that the effect of a single polymorphic gene need not be significant or can be dependent on the genetic background.

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Received: 02–09–06
Accepted after corrections: 03–02–18

ABSTRAKT

Výzkum polymorfismů v genu myostatinu (*GDF8*; *MSTN*) u prasat

Myostatin je růstový faktor, který během embryonálního vývoje řídí proliferaci myoblastů. Mutace v kódujících sekvencích genu myostatinu (*GDF8*) u skotu vedou ke vzniku hyperplazie, což ukazuje na jeho inhibiční funkci vzhledem k proliferaci myoblastů. Cílem této studie bylo vyhledat mutace v oblasti promotoru (397 bp), exonu 2 s přilehlými oblastmi (631 bp) a exonu 3 s přilehlými oblastmi (899 bp) v genu *GDF8* (MSTN) u prasat s použitím analytických metod PCR-RFLP, SSCP a DSCP. Ke stanovení restrikčního polymorfismu v oblasti promotoru *GDF8* byly použity enzymy *MnlI* a *DraI*. V polymorfním místě *DraI* měla tři prasata genotyp *AT* a 291 prasat mělo genotyp *TT*. Při použití enzymu *MnlI* nebyl restrikční polymorfismus zjištěn. Analýzou SSCP v 12% polyakrylamidovém gelu nebyly zjištěny žádné rozdíly v rychlosti migrace mezi produkty PCR. Ani analýza SSCP 30 produktů PCR a analýza DSCP 14 produktů PCR zahrnujících exon 2 nevedla ke zjištění žádných mutací. Na polymorfismus *TaqI* v exonu 3 bylo analyzováno celkem 314 prasat; z tohoto počtu 13 jedinců byli homozygoti *TT*, 112 heterozygoti *CT* a 189 homozygoti *CC*. V této oblasti nebyl zaznamenán žádný polymorfismus SSCP nebo DSCP. Obecně se potvrzuje, že gen myostatinu u prasat je konzervovanější než u skotu. Dodatečné statistické porovnání některých jatečných znaků mezi jedinci *CC* a *CT* nevykazovalo statisticky významné rozdíly.

Klíčová slova: gen myostatinu; *GDF8*; *MSTN*; prase; mutace; polymorfismus

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The influence of rape seed/oil on the quality of chicken carcasses

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ABSTRACT: The aim of the study was to investigate the effect of replacement of swine lard by rape oil and seed in broiler diets on carcass quality, fat deposition and composition of fatty acids in the lipids of breast muscles and abdominal fat. The study was performed on 72 carcasses of Ross 208 male broilers. Regarding the feeding regime, broilers were divided into three groups: in the finisher, the first group was given 7.5% swine lard, together with other feeds; the 2nd group was given 6.2% rape oil and the 3rd group received 13.5% rape seed and 2% swine lard. The following carcass weights were found in the three groups of chicken: 1 944 g, 1 892 g and 1 826 g, respectively. The addition of rape products to broiler diets resulted in a decrease in carcass weights ($P < 0.01$) and had a significant influence on the yield of wings ($P < 0.01$) and back ($P < 0.05$) in the carcass and on the yield of skin and subcutaneous fat in the breast ($P < 0.01$) as well as of muscle and skin with subcutaneous fat ($P < 0.05$) in the carcass. It significantly affected ($P < 0.01$) the difference between the deposition of abdominal adipose tissue in broilers of the 1st and 2nd as well as the 2nd and 3rd group. The addition of rape oil and seed to broiler diets lowered the content of SFA, but increased the content of MUFA and α-linolenic fatty acid in muscles and in abdominal fat. The differences in linoleic (C18 : 2 n-6) and α-linolenic (C18 : 3 n-3) fatty acids were statistically significant ($P < 0.01$). At the same time, PUFA n-6 and PUFA n-3 ratio was lowered from 21.26 to 6.63 and 7.03 in the lipids of breast muscles, and from 16.34 to 7.62 and 8.17 in the lipids of abdominal fat.

Keywords: chicken; carcass; rape oil/seed; fatty acids; polyunsaturated fatty acids

Although chicken meat is considered to be a dietetic product, new technologies are developed that alter its nutritive composition in order to reduce the cholesterol level and change the ratio of essential fatty acids. These technologies are expected to have positive impacts on human health. Polyunsaturated fatty acids, especially those from n-3 group, become very important for nutritionists because they play a significant role in prevention of stress induced diseases and of those induced by improper diets (Barlow and Pike, 1991; Albrecht and Klein, 1995). Polyunsaturated n-3 fatty acids decrease the risk of heart diseases and psoriasis. Moreover, they are necessary for normal development of brain and nerve tissue (Leaf and Weber, 1988; Barlow and Pike, 1991). Altering the fat composition in broiler diets by inclusion of some feeds results in so called "designed" meat, rich in n-3 polyunsaturated fatty acids, such as α-linolenic (C18 : 3 n-3), eicosapentaenoic (C20 : 5 n-3) and docosahexaenoic (C22 : 6 n-3), as stated by Haumann (1993).

Plant sources of fats, rich in n-3 fatty acids, are added to broiler diets in order to improve the fatty acid profile of meat and eggs, keeping in mind the satisfactory flavour of the same product (Chanmugam *et al.*, 1992; Ajuyah *et al.*, 1993). The seed of flax and some grasses is rich in α-linolenic and linoleic fatty acids while sunflower seed is rich in linoleic acid. Rape seed contains considerable amounts of α-linolenic and linoleic fatty acids too, therefore it is used as a source of protein and energy for broiler diets in many countries. The possibilities of increasing the α-linolenic fatty acid content using rape products were demonstrated by researches of Zollitsch *et al.* (1993), Lettner and Zollitsch (1993), Kralik *et al.* (1997) and Lopez-Ferrer *et al.* (1997).

Linoleic acid, LA (C18 : 2 n-6) and α-linolenic (αLNA) are not synthesised in higher animals but

only in plants. In metabolism of linoleic acid, the chain is desaturated and elongated into μ-linolenic acid and arachidonic acid, AA (C20 : 4 n-6), while αLNA is metabolised into eicosapentaenic (EPA) and docosahexaenic acid (DHA). The possibilities of alteration of acids from n-6 to n-3 and vice versa do not exist. For this reason, tissues with polyunsaturated fatty acids vary a lot regarding their composition (n-6/n-3 ratio), in respect to the selection of feeds in the diet. The PUFA n-6/PUFA n-3 ratio in fatty tissue influences many aspects of animal physiology including behaviour and health status. Consequently, there is an influence on human health as well. New findings from western industrialised countries point out the fact that not cholesterol but the longer intake of LA (n-6) with relative "deficiency" of n-3 acids is the main risk factor of cancer, coronary diseases (CHD), cerebrovascular diseases (CVD) and allergic hyperactivity. Therefore, it is important to reduce the n-6/n-3 acids ratio in meat and milk by using particular feeds in diets (Okuyama and Ikemoto, 1999).

The aim of our study was to investigate the effect of replacement of swine lard by rape seed/oil on carcass traits of broilers as well as on the fatty acid profile in lipids of breast muscles and abdominal fat.

MATERIAL AND METHODS

The study was performed on 72 male broilers of Ross-208 provenience. Broilers were divided into three equal groups. In the first period of fattening, until 21st day, all groups of broilers were fed diet A that contained 22.00% crude protein and 13.08 MJ/kg AME_n. From 22nd to 49th day of fattening three groups of broilers received diets B that contained 20.67%, 20.59% and 20.51% crude protein, respectively, and 13.00 MJ/kg, 12.76 MJ/kg and 12.89 MJ/kg AME_n, respectively (Table 1). Differences between the groups were in the source of lipids. The first group was given a finisher with 7.5% of swine lard, 2nd group 6.2% rape oil, and

Table 1. Composition of the diets (%)

Ingredient	Diet A (starter)	Diets B (finisher)		
		1st group	2nd group	3rd group
Corn	44.1	47.6	49.4	45.6
Soybean meal	43.0	37.0	36.5	31.0
Sunflower meal		3.0	3.0	3.0
Rape oil	–	–	6.2	–
Full-fat rapeseed	–	–	–	13.5
Animal fat – swine lard	8.0	7.5	–	2.0
Limestone	1.8	1.8	1.8	1.8
Phosphonal (18% P)	2.3	2.3	2.3	2.3
Salt	0.3	0.3	0.3	0.3
Premix	0.5	0.5	0.5	0.5
Calculated nutrient content				
Crude protein	22.02	20.67	20.59	20.51
Fat	9.87	9.43	8.18	8.92
Ash	7.34	7.19	7.18	6.09
Lysine	1.29	1.16	1.16	1.20
Methionine + cysteine	0.70	0.68	0.68	0.68
Ca	0.98	0.97	0.96	0.93
P	0.81	0.81	0.81	0.63
AME_n (MJ/kg)	13.08	13.00	12.76	12.89

Phosphonal (18% P) – supplement containing 18% phosphorus
AME_n – Apparent Metabolic Energy

Table 2. Fatty acids in diets (% of total fatty acids)

Fatty acid	Diet A (starter)	Diets B (finisher)		
		1st group	2nd group	3rd group
Myristic (C14 : 0)	1.0	1.0	0.3	0.3
Palmitic (C16 : 0)	20.0	16.6	7.1	11.0
Palmitoleic (C16 : 1)	2.0	4.6	0.5	0.4
Stearic (C18 : 0)	11.0	8.3	1.8	2.8
Oleic (C18 : 1)	38.0	37.0	49.0	48.0
Linoleic (C18 : 2 n-6)	25.0	29.5	34.8	32.0
α-linolenic (C18 : 3 n-3)	1.5	1.7	4.8	4.4
Eicosenoic (C20 : 1)	0.8	0.6	1.0	0.7
Arachidonic (C20 : 4 n-6)	0.1	0.1	–	–
Erucic (C22 : 1 n-9)	0.1	0.1	0.2	0.1
Saturated (SFA)	32.0	25.9	9.2	14.1
Monounsaturated (MUFA)	40.73	42.29	50.62	48.03
Polyunsaturated (PUFA n-6)	25.11	29.64	34.81	32.04
Polyunsaturated (PUFA n-3)	1.52	1.73	4.81	4.45
PUFA n-6/PUFA n-3	16.52	17.13	7.52	7.20

3rd group 13.5% rape seed and 2% swine lard. The profile of fatty acids in the diet is given in Table 2.

After slaughter, broiler carcasses were cut into main parts: breasts, thighs with drumsticks, back and wings. Abdominal fat was carefully separated from each carcass. The breasts and thighs with drumsticks were dissected into muscle tissue, bones and skin with fatty tissue. The main parts are given as the percentage of the carcass, individual tissues as the percentage of the main part and as the percentage of total carcass.

The fat content was determined by Soxhlet method. The fatty acid composition in diets, in the muscle and in abdominal fatty tissue (n = 10 per group) was determined by Chrompack CP 9000 chromatograph equipped with a flame ionisation detector. The following fatty acids were determined: C14 : 0, C16 : 0, C17 : 0, C18 : 0, C16 : 1, C18 : 1, C20 : 1, C18 : 2 n-6, C18 : 3 n-3, C20 : 4 n-6 and C22 : 1 n-9. Some of the fatty acids were given as the percentage of all fatty acids in the sample. The results of the investigations of particular traits were presented by arithmetic mean and standard deviation ($\bar{x} \pm s$); the influence of the treatment was investigated by analysis of variance, and differences between the groups of broilers were tested by t-test. Statistical data were processed on a personal computer using Statistica v.5.0 for Windows software.

RESULTS AND DISCUSSION

The investigation showed that carcass yield (Table 3) was higher in the 1st group when compared to the 2nd and 3rd group of broilers, but the differences were not significant ($P > 0.05$). The highest carcass weight (Table 4) was found in broilers from the 1st group (1 944 g), which is on average 2.70% and 6.09%, respectively, more than carcass weights of broilers from the 2nd and 3rd group,. The inclusion of rape products (oil and seed) lowered the carcass weight. The differences between investigated groups were observed between 1st and 3rd group in carcass weights ($P < 0.01$) as well as between 1st and 2nd group in the proportion of wings ($P < 0.01$) and back ($P < 0.05$) in the carcass.

Decreased weights of broilers fed rape seed were also reported by Ajuyah *et al.* (1991), Mawson *et al.* (1994), Roth-Maier and Kirchgessner (1995). It was explained by the presence of antinutritive factors. Krasicka *et al.* (2000) obtained higher carcass weights of broilers on diets with rape oil included as the source of fat.

Fatty tissue deposition in the abdominal cavity was highest in broiler carcasses from the 1st group (2.34%), followed by 3rd and 2nd group: 2.10% and 1.61%, respectively. The differences in abdomi-

Table 3. Effect of diet on the carcass characteristics of broilers

Indicator	1st group (n = 24)	2nd group (n = 24)	3rd group (n = 24)	A	B	C
Carcass yield (%)	75.25 ± 2.32	74.92 ± 1.25	74.52 ± 1.48	n.s.	n.s.	n.s.
Abdominal fat (%)	2.34 ± 0.60	1.61 ± 0.56	2.10 ± 0.64	**	n.s.	**

A = $\bar{x}_1 - \bar{x}_2$; B = $\bar{x}_1 - \bar{x}_3$; C = $\bar{x}_2 - \bar{x}_3$
**$P < 0.01$; n.s. $P > 0.05$

Table 4. Proportions of main parts in broiler carcasses

Indicator	1st group (n = 24)	2nd group (n = 24)	3rd group (n = 24)	A	B	C
Carcass weight (g)	1944.10 ± 144.32	1892.15 ± 198.25	1826.20 ± 153.10	n.s.	**	n.s.
Breast (%)	26.04 ± 1.62	26.73 ± 1.01	26.05 ± 1.72	n.s.	n.s.	n.s.
Thighs with drumsticks (%)	30.68 ± 1.22	31.03 ± 1.04	30.81 ± 1.61	n.s.	n.s.	n.s.
Back (%)	29.68 ± 1.05	29.05 ± 1.11	29.57 ± 1.26	*	n.s.	n.s.
Wings (%)	11.26 ± 0.33	11.58 ± 0.33	11.47 ± 0.58	**	n.s.	n.s.

A = $\bar{x}_1 - \bar{x}_2$; B = $\bar{x}_1 - \bar{x}_3$; C = $\bar{x}_2 - \bar{x}_3$
**$P < 0.01$; *$P < 0.05$; n.s. $P > 0.05$

nal fat proportions in the carcasses were statistically significant ($P < 0.01$) between 1st and 2nd as well as between 2nd and 3rd group.

The proportions of the most valuable parts, such as breasts and thighs with drumsticks, were highest in the carcasses of broilers from the 2nd group (57.76%), followed by the broilers from the 3rd (56.86%) and 1st group (56.72%).

Tables 5 and 6 show relative proportions of tissues in the main parts and in the carcass. Between the 1st and 2nd group of broilers fed diets in which swine lard was substituted by rape oil and rape seed, statistically significant difference was observed in the proportion of skin and subcutaneous fat of breast ($P < 0.01$). The proportion of muscle and skin with subcutaneous fat of breast in the carcass differed between 1st and 2nd group ($P < 0.05$). No statistically significant differences were found either in the proportions of individual tissues in thighs with drumsticks nor in the proportions of these tissues in carcass ($P > 0.05$). It was also found in our previous researches (Kralik *et al.*, 1996).

The results of research on fatty acids in the lipids of breast muscles (Table 7) showed that saturated fatty acids (SFA) were present at a higher amount (32.44%) in the 1st group of broilers, which received swine lard in the diet, than in the 2nd and 3rd group (24.97% and 23.69%, respectively). Simultaneously, the amount of monounsaturated fatty acids (MUFA) such as C16 : 1, C18 : 1 and C20 : 1 increased from 37.59% in the 1st group to 46.38% and 45.89% in the 2nd and 3rd group of broilers, respectively. The amount of C18 : 2 n-6 and C20 : 4 n-6 in the lipids of breast muscles was significantly different between the groups of broilers ($P < 0.01$).

The replacement of swine lard by dietary rape products caused a statistically significant decrease in stearic and palmitic acid, but α-linolenic acid ($P < 0.01$) in the lipids of breast muscular tissue of the broilers increased. These results are in accordance with results reported by Holsheimer (1991), Zollitsch *et al.* (1993) and Kralik *et al.* (1996, 1997). Polyunsaturated (PUFA) eicosapentaenoic (C20 : 5 n-3) and docosahexaenoic (C22 : 6 n-3) fatty acids were found in traces in all investigated groups of broilers.

The PUFA n-6/PUFA n-3 ratio was more favourable in the lipids of breast muscles in the 2nd and 3rd group (6.63 and 7.03, respectively) than in the

Table 5. Tissues in breast and carcass

Tissue	1st group (n = 24)	2nd group (n = 24)	3rd group (n = 24)	A	B	C
In breast (%)						
Muscle	72.61 ± 2.55	74.26 ± 3.10	73.65 ± 3.03	n.s.	n.s.	n.s.
Skin and subcutaneous fat	11.12 ± 1.45	9.70 ± 2.03	10.40 ± 2.48	**	n.s.	n.s.
Bones	16.27 ± 2.59	16.04 ± 1.98	15.96 ± 1.94	n.s.	n.s.	n.s.
In carcass (%)						
Muscle	18.92 ± 1.52	19.85 ± 1.11	19.20 ± 1.62	*	n.s.	n.s.
Skin and subcutaneous fat	2.88 ± 0.34	2.59 ± 0.53	2.71 ± 0.70	*	n.s.	n.s.
Bones	4.23 ± 0.71	4.29 ± 0.60	4.14 ± 0.46	n.s.	n.s.	n.s.

A = $\bar{x}_1 - \bar{x}_2$; B = $\bar{x}_1 - \bar{x}_3$; C = $\bar{x}_2 - \bar{x}_3$

**P < 0.01; *P < 0.05; n.s. P > 0.05

Table 6. Tissues in thighs with drumsticks and carcass

Tissue	1st group (n = 24)	2nd group (n = 24)	3rd group (n = 24)	A	B	C
In thighs with drumsticks (%)						
Muscle	64.61 ± 2.76	64.68 ± 2.34	64.61 ± 2.94	n.s.	n.s.	n.s.
Skin and subcutaneous fat	12.28 ± 2.19	12.20 ± 1.31	11.88 ± 1.91	n.s.	n.s.	n.s.
Bones	23.11 ± 1.73	23.12 ± 2.40	23.51 ± 2.11	n.s.	n.s.	n.s.
In carcass (%)						
Muscle	19.81 ± 1.04	20.01 ± 0.92	19.91 ± 1.47	n.s.	n.s.	n.s.
Skin and subcutaneous fat	3.77 ± 0.73	3.79 ± 0.63	3.67 ± 0.66	n.s.	n.s.	n.s.
Bones	7.09 ± 0.63	7.24 ± 0.79	7.23 ± 0.57	n.s.	n.s.	n.s.

A = $\bar{x}_1 - \bar{x}_2$; B = $\bar{x}_1 - \bar{x}_3$; C = $\bar{x}_2 - \bar{x}_3$

n.s. P > 0.05

1st group (21.26), which means that the inclusion of rape products in broiler diets favourably affected the alteration of this ratio in the breast muscle lipids.

The results of research on SFA in the abdominal fat of broilers (Table 8) show that the replacement of swine lard by rape seed in broiler diets decreased an SFA amount from 30.93% to 20.53% and 19.90%, respectively. At the same time, the percentage of MUFA increased from 45.43% to 52.68% and 50.98%, respectively. The highest proportion of oleic acid was found in the 2nd group (48.75%) and the lowest in abdominal fat of the 1st group of broilers (40.97%). The highest level of linoleic acid was found in broilers of the 3rd group (25.05%); followed by broilers from the 2nd and 1st group (22.68% and 21.02%), respectively. In comparison with the 1st group, the increase in α-linolenic acid content in the abdominal fat of broilers was higher by 130% and 138%, respectively, in the 2nd and 3rd group. From the aspect of human nutrition, the results point out the fact that the use of dietary rape oil and full-fat rape seed results in the more favourable fatty acid composition in broiler carcasses. This conclusion is in accordance with published results of the authors who already dealt with similar re-

Table 7. Profile of fatty acids in breast muscle (% of total fatty acids)

Fatty acid	1st group	2nd group	3rd group	A	B	C
Myristic (C14 : 0)	1.63 ± 0.07	0.89 ± 0.06	0.91 ± 0.06	**	**	n.s
Palmitic (C16 : 0)	19.63 ± 0.48	14.84 ± 0.51	14.77 ± 0.55	**	**	n.s.
Palmitoleic (C16 : 1)	2.18 ± 0.65	1.74 ± 0.50	2.03 ± 0.58	n.s.	n.s.	n.s.
Heptadecanoic (C17 : 0)	0.22 ± 0.11	0.18 ± 0.08	0.30 ± 0.73	**	n.s.	n.s.
Stearic (C18 : 0)	10.96 ± 1.05	9.06 ± 0.09	7.71 ± 0.08	**	**	**
Oleic (C18 : 1)	35.08 ± 0.98	44.25 ± 0.75	43.36 ± 0.82	**	**	*
Linoleic (C18 : 2 n-6)	23.75 ± 0.50	20.63 ± 0.40	21.79 ± 0.35	**	**	*
α-linolenic (C18 : 3 n-3)	1.29 ± 0.11	3.59 ± 0.10	3.60 ± 0.09	**	**	n.s.
Eicosenoic (C20 : 1)	0.33 ± 0.02	0.39 ± 0.01	0.50 ± 0.01	*	**	**
Arachidonic (C20 : 4 n-6)	3.68 ± 0.05	3.19 ± 0.04	3.55 ± 0.03	**	**	**
Saturated (SFA)	32.44	24.97	23.69			
Monounsaturated (MUFA)	37.59	46.38	45.89			
Polyunsaturated (PUFA n-6)	27.43	23.82	25.34			
Polyunsaturated (PUFA n-3)	1.29	3.59	3.60			
PUFA n-6/PUFA n-3	21.26	6.63	7.03			

A = $\bar{x}_1 - \bar{x}_2$; B = $\bar{x}_1 - \bar{x}_3$; C = $\bar{x}_2 - \bar{x}_3$

**$P < 0.01$; *$P < 0.05$; n.s. $P > 0.05$

Table 8. Profile of fatty acids in abdominal fat (% of total fatty acids)

Fatty acid	1st group	2nd group	3rd group	A	B	C
Myristic (C14 : 0)	1.13 ± 0.08	0.71 ± 0.07	0.72 ± 0.13	**	**	n.s.
Palmitic (C16 : 0)	21.81 ± 0.56	14.45 ± 1.08	14.48 ± 1.22	**	**	n.s.
Palmitoleic (C16 : 1)	3.96 ± 0.69	3.35 ± 0.93	3.83 ± 1.19	n.s.	n.s.	n.s.
Heptadecanoic (C17 : 0)	0.30 ± 0.10	0.25 ± 0.10	0.32 ± 0.14	n.s.	n.s.	n.s.
Stearic (C18 : 0)	7.69 ± 1.07	5.12 ± 0.85	4.38 ± 0.78	**	**	n.s.
Oleic (C18 : 1)	40.97 ± 0.99	48.75 ± 1.18	46.59 ± 1.27	**	**	**
Linoleic (C18 : 2 n-6)	21.02 ± 0.51	22.68 ± 0.86	25.05 ± 1.44	**	**	**
α-Linolenic (C18 : 3 n-3)	1.30 ± 0.17	3.00 ± 0.35	3.09 ± 0.25	**	**	n.s.
Eicosenoic (C20 : 1)	0.50 ± 0.03	0.58 ± 0.06	0.56 ± 0.08	**	*	n.s.
Arachidonic (C20 : 4 n-6)	0.22 ± 0.08	0.18 ± 0.06	0.20 ± 0.05	n.s.	n.s.	n.s.
Saturated (SFA)	30.93	20.53	19.90			
Monounsaturated (MUFA)	45.43	52.68	50.98			
Polyunsaturated (PUFA n-6)	21.24	22.86	25.25			
Polyunsaturated (PUFA n-3)	1.30	3.00	3.09			
PUFA n-6/PUFA n-3	16.34	7.62	8.17			

A = $\bar{x}_1 - \bar{x}_2$; B = $\bar{x}_1 - \bar{x}_3$; C = $\bar{x}_2 - \bar{x}_3$

**$P < 0.01$; *$P < 0.05$; n.s. $P > 0.05$

search subjects (Zollitsch *et al.*, 1993; Lettner and Zollitsch, 1993; Lopez-Ferrer *et al.*, 1997; Kralik *et al.*, 1996, 1997).

The addition of rape products increased the amount of C18 : 1 and C18 : 3 n-3, but it also resulted in a significant decrease of C16 : 0 and C18 : 0 percentages. These changes are in accordance with the results reported by Ajuyah *et al.* (1991) for thighs and Yau *et al.* (1991) for the breast muscles of broilers that received rape seed and rape oil or olive oil as the source of predominantly monounsaturated fatty acid. Yau *et al.* (1991) reported that the effect of dietary fat on the lipid composition of breast muscle was less pronounced than for abdominal fat.

In this study, the ratio PUFA n-6/PUFA n-3 in abdominal fat of broilers decreased from 16.34 (1st group) to 8.17 and 7.62 (3rd and 2nd group, respectively) as a consequence of n-6/n-3 fatty acid ratio alteration in broiler diets. This confirms a possibility of successfully "designing" the products that better meet the requirements for modern human nutrition from the nutritive and physiological aspect. The fatty acid composition of breast muscles and abdominal fat mainly coincided with the differences in dietary fatty acid intake. These findings agree with those of Ajuyah *et al.* (1991) and Scaife *et al.* (1994).

CONCLUSION

From the study on the influence of different fat sources in diets for broilers (1st group 7.5% of swine lard, 2nd group 6.2% rape oil, and 3rd group 13.5% rape seed and 2% swine lard), the following conclusions can be drawn:

- The following carcass weights were found in the three groups of chickens: 1 944 g, 1 892 g and 1 826 g, respectively. The addition of rape oil and seed to diets for broilers significantly influenced carcass weights of broilers in the 1st and 3rd group ($P < 0.01$) and the percentages of wings ($P < 0.01$) and back ($P < 0.05$) in carcass.
- Fat deposition in abdomen was highest in broilers of the 1st group (2.34%), followed by the 3rd (2.10%) and 2nd group (1.61%). Differences in abdominal fat proportions in broiler carcasses were statistically significant ($P < 0.01$) between 1st and 2nd group as well as between 2nd and 3rd group.
- The alteration of fatty acid profiles in dietary lipids resulted in the change of fatty acid composition in broiler carcasses. The use of rape oil and seed in broiler diets increased α-linolenic acid content in the lipids of breast muscles by 101% and 106%, while in abdominal fat this increase was 130% and 138%, respectively. Differences in the contents of linoleic acid (C18 : 2 n-6) and α-linolenic acid (C18 : 3 n-3) were statistically significant ($P < 0.01$).
- The PUFA n-6/PUFA ratio in the lipids of breast muscles was changed from 21.26% (1st group) to 6.63% (2nd group) and 7.03% (3rd group) while in abdominal fat the same ratio of n-3 fatty acids was altered from 16.34 (1st group) to 7.62 (2nd group) and 8.11 (3rd group), which is more favourable from the aspect of human nutrition.

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Received: 01–11–12
Accepted after corrections: 03–01–08

ABSTRAKT

Vliv řepkového semene nebo oleje na kvalitu jatečných trupů u kuřat

Cílem studie bylo zjistit vliv náhrady vepřového sádla v krmných směsích pro brojlery řepkovým olejem a řepkovým semenem na kvalitu jatečně opracovaného těla, na ukládání tuku a na zastoupení mastných kyselin v lipidech prsní svaloviny a abdominálního tuku. Šetření jsme prováděli u 72 kohoutků hybridní kombinace Ross 208. Pro výkrm jsme kuřata rozdělili do tří skupin: první skupina dostávala ve finišeru 7,5 % vepřového sádla, druhá skupina 6,2 % řepkového oleje a třetí skupina 13,5 % řepkového semene a 2 % vepřového sádla. Hmotnost jatečně opracovaného trupu byla 1 944 g, 1 892 g a 1 826 g. Přídavek řepkových produktů do směsí pro brojlery tedy vedl k poklesu hmotnosti jatečného trupu ($P < 0,01$) a měl významný vliv také na procentuální podíl křídel ($P < 0,01$), hřbetu, svaloviny a kůže s podkožním tukem z jatečně opracovaného trupu ($P < 0,05$) a na procentuální podíl kůže a podkožního tuku z prsou ($P < 0,05$). Významně ($P < 0,01$) ovlivnil rozdíl v ukládání abdominální tukové tkáně u brojlerů 1. a 2. skupiny, jakož i 2. a 3. skupiny. Přídavek řepkového oleje a semene do směsí pro brojlery snížil obsah nasycených mastných kyselin, ale zvýšil obsah mononenasycených mastných kyselin a kyseliny α-linolenové ve svalovině a v abdominálním tuku. Rozdíly v obsahu kyseliny linolové (C18 : 2 n-6) a α-linolenové (C18 : 3 n-3) byly statisticky významné ($P < 0,01$). Zároveň se snížil poměr polynenasycených mastných kyselin n-6 a n-3 v lipidech prsní svaloviny z 21,26 na 6,63 a 7,03 a v lipidech abdominálního tuku z 16,34 na 7,62 a 8,17.

Klíčová slova: kuře; jatečně opracovaný trup; řepkový olej; řepkové semeno; mastné kyseliny; polynenasycené mastné kyseliny

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Effect of sex, growth intensity and heat treatment on fatty acid composition of common carp (*Cyprinus carpio*) fillets

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ABSTRACT: Forty-eight individuals of common carp were selected in the third year of life among fish reared in an earthen pond and supplementarily fed wheat grain. Live weight of the fish ranged from 1 172 to 3 196 grams. Fatty acid (FA) content in fish fillets without skin was determined by gas chromatography after the extraction of total lipids with a hexane/2-propanol mixture. Sexual dimorphism in chemical composition of marketable carp meat was insignificant ($P > 0.05$) at the time of autumn harvest. Dry matter and intramuscular fat content in fillets increased linearly with the increasing growth rate while protein content decreased ($P < 0.05$). The percentage of saturated and monounsaturated FA did not change ($P > 0.05$) and increased ($P < 0.001$), respectively. That of polyunsaturated FA (PUFA) decreased ($P < 0.01$), a decrease in n-3 PUFA being faster in comparison with n-6 PUFA. Stewing decreased ($P < 0.05$) C16 : 0, C22 : 5 n-3 and C22 : 5 n-6 content and increased the content of C20 : 1 ($P < 0.01$).

Keywords: *Cyprinus carpio*; fatty acids; growth; heat treatment of meat; sexual dimorphism

Fatty acid composition of fish reflects that of the diet to a large extent. Marketable carp from natural waters exhibited high concentrations of linoleic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) while carp fed diets rich in carbohydrates showed a high level of oleic acid (OA, C18 : 1 n-9) in muscles (Farkas *et al.*, 1978; Csengeri *et al.*, 1978; Watanabe *et al.*, 1981; Runge *et al.*, 1987; Schwarz *et al.*, 1988).

PUFA content in carp meat was reported to be in a very wide range: 11.6–15.7% of total fatty acids (depending on the culture line and pond type; Bieniarz *et al.*, 2000) to 32.3–34.5% (Geri *et al.*, 1995b). The same is true regarding the ratio of n-3 to n-6 PUFA: 1.12 (Vácha and Tvrzická, 1995) to 3.02 (Sýkora and Valenta, 1978). Muscles of common carp reared in warm water showed a higher n-3 to n-6 PUFA ratio (1.52) in comparison with carp of the same age reared in water of natural temperature (0.47; Geri *et al.*, 1995b).

Percentage of particular fatty acids can be influenced by changes in live weight (Geri *et al.*, 1995a). On the other hand, the effect of sex on the FA content is not described in the available literature.

The effect of heat treatment of fish tissue on the FA content was studied by many authors. Quaglia *et al.* (1974) found significant changes in the spectrum of FA after either baking or boiling in several fish species. Myers and Harris (1975) found no effect of heat treatment (electronic and conventional heating) on the FA composition of meats. Gall *et al.* (1983) concluded that the FA composition of fish fillets is not significantly changed by baking, broiling, or microwave cooking. Maeda *et al.* (1985) found no changes in FA composition in total lipids of sardines after grilling or broiling. Tothmarkus and Sasskiss (1993) mentioned no significant difference in the fatty acid composition of raw and boiled fish, contents of PUFA, EPA and DHA were surprisingly stable.

The objective of the present experiment was to assess the effect of growth rate, sex, and heat treatment on the content of fatty acids, total lipids and crude protein in fillets of common carp (*Cyprinus carpio*) from the autumn harvest.

Supported by the Grant Agency of the Czech Republic (Project No. 525/01/P078) and Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 432100001).

MATERIAL AND METHODS

On 29 March 2001, six hundred 2-year-old carps with the mean live weight of 558 ± 4.6 g (mean ± standard error of the mean) were released into an earthen pond with an area of 1.17 hectares. The fish received supplementary feed based on wheat grain. The harvest was carried out on 9 October 2001, i. e. immediately after the end of the feeding period. Altogether 120 individuals with live body weight ranging from 1 172 to 3 196 g were selected. Animals were individually weighed, killed and sexed. After the determination of their sex two groups of 24 males and 24 females were selected with the objective to obtain the maximum variance in body weight (fish of the same age but with very different growth rates were used). Selected animals were filleted, both fillets were flayed, weighed and frozen for further analyses. Thereafter one fillet was ground in a Moulinex blender and used for chemical analyses. The second fillet of 12 males and 12 females was rolled and placed into a stewing glass bottle. A digital thermometer was placed in the middle of each roll. Bottles were placed into a thermostat set up to 200°C. Stewing was finished when the inner temperature of the sample reached 80°C. Samples were left at room temperature for 30 minutes. During this time interval, the temperature inside the glass remained practically unchanged. Thereafter the samples were quickly cooled, ground and used for analyses.

Chemical analyses

Total nitrogen (N) was determined according to Czech Standard CSN 57 0185 using Kjeltec 2300 (Tecator, Sweden). Crude protein content was calculated using the factor pertinent to meat: N × 6.0. Total lipids were determined gravimetrically after extraction by the modified method of Hara and Radin (1978) using a hexane/2-propanol (HIP) mixture. HIP extract was used for fatty acid determinations.

Extraction of total lipids from meat by means of HIP

The following solvents were used: hexane (p.a. 99.0%, ACS, Merck, Darmstadt, Germany), 2-propanol (p.a., 99.7%, Dorapis, Prague, Czech Republic) 3 : 2, v/v (HIP1) and 7 : 2, v/v (HIP2), respectively. 35 g of meat was homogenized with 180 ml HIP1 for 1 minute in a Diax 900 disintegrator (Heidolph, Germany) at 10 000 rpm and filtered through the Büchner funnel. The disintegrator, funnel, and the sample residue were washed three times with 15-ml portions of HIP1, the residue being resuspended each time. 120 ml of 0.4694 mol/l aqueous Na_2SO_4 was added. After shaking in the separating funnel and separation of the layers, the water layer was re-extracted with 50 ml of HIP2. The separated hexane layer was passed through anhydrous Na_2SO_4 to a 250 ml volumetric flask and filled up to the mark with hexane. A 30ml aliquot was taken for gas chromatographic (GC) analysis. The rest of the solution was evaporated on a rotary vacuum evaporator RVO 200A model (Ingos, Prague, Czech Republic) at 40°C. Evaporation was finished under nitrogen. Total lipids were determined gravimetrically.

Fatty acid determination

The method of Komprda *et al.* (1999) was used for FA determination.

An aliquot (40–60 mg; exactly measured) of the cleaned HIP extract was evaporated to dryness under nitrogen. The solid residue was weighed in the reaction flask and 2 ml of isooctane (99.5%, p.a. ACS, Merck, Darmstadt, Germany) with the internal standard (2.5 mg/ml pentadecanoic acid in isooctane, 99.0% C15 : 0, Sigma-Aldrich, St. Louis, USA). 2 ml of 0.5 mol/l methanolic solution of CH_3ONa (11.5 g/l Na in CH_3OH) was added after ultrasonication and the mixture was boiled for 5 minutes under Dimroth reflux. 2 ml of 14% solution of BF_3 in CH_3OH was added through the condenser and the mixture was refluxed for another 5 minutes. The heating was removed, 2 ml of isooctane were added, the mixture was shaken and left to stand for 1 minute. 5 ml of saturated aqueous solution of NaCl was added and the mixture was shaken vigorously for 15 seconds while tepid. The organic layer was transferred into the test vial and 1 µl was injected by split injector (Agilent Technologies, Folsom, California, USA) into the gas chromatographic column.

Fatty acid methyl esters were separated using gas chromatograph HP 6890 A (Hewlett – Packard, Palo Alto, CA, USA) equipped with flame ionisa-

tion detector (FID) and capillary column Inowax 19091N-233 (30 m × 0.25 mm × 0.5 µm, Agilent Technologies, Folsom, California, USA) with the temperature programme 205°C held for 9 min, ramp 5°C/min up to 240°C, held at 240°C for 19 min, ramp 10°C/min up to 250°C and held at 250°C for 13 min. The injector temperature was 280°C and the detector temperature 300°C. The flow rate of the carrier gas (N_2, Siad, 99.999%) was 1.0 ml/minute.

The following fatty acids were determined in fish meat: C14 : 0, C16 : 0, C18 : 0, C16 : 1, C18 : 1, C18 : 2 n-6, C18 : 3 n-6, C20 : 1, C20 : 4 n-6, C22 : 4 n-6, C22 : 5 n-6, C18 : 3 n-3, C20 : 5 n-3, C22 : 5 n-3 and C22 : 6 n-3. A mixture of 37 methyl ester standards of fatty acids Supelco 37 Component FAME mix (Supelco, Sigma-Aldrich, St. Louis, MO, USA) was used for qualitative evaluation. The content of the particular fatty acids was expressed as a per cent of the sum of all analysed fatty acids.

The statistical analyses were performed according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Growth markers

Losses of fish were 3.7%, the average weight gain was 1 342 g and the feed consumption per unit gain was 2.45. The live weight of fish was 2 044 ± 66.9 g. Females tended ($P > 0.05$) to be heavier than males (2 164 ± 96.9 and 1 923 ± 87.3 g, resp.).

Chemical analyses

The use of HIP followed by a wash of the extract with aqueous sodium sulfate to remove nonlipid contaminants has a number of advantages over the common usage of chloroform : methanol. The solvents are less toxic and cheaper, interference in processing by proteolipid protein contamination is avoided, the two phases separate rapidly during the washing step, etc. (Hara and Radin, 1978).

Effect of sex on fillet composition

Sex differences in dry matter, crude protein and total lipid content (Table 1), and fatty acid content (Table 2) were insignificant ($P > 0.05$) at the time of autumn harvest.

Considering both sexes as a one set, the mean value of dry matter, crude protein and HIP extract was 264.3 ± 2.30, 157.4 ± 0.88 and 80.1 ± 2.63 g per kg, respectively.

Effect of growth intensity

The growth rate significantly influenced meat composition. Dry matter content in the meat of fish

Table 1. Dependence of meat composition on live weight of carp

Component – Y	Mean ± standard error of the mean [1]	$Y = a + b X$ [1]				Females[3]	Males[3]
		a	b	r	P[2]	mean ± standard error of the mean	
Dry matter (g/kg)	264.3 ± 2.30	242.2	0.0108	0.315	<0.05	264.9 ± 3.69	263.7 ± 2.82
Crude protein (N × 6) (g/kg)	157.4 ± 0.88	167.0	–0.0047	0.359	<0.05	157.2 ± 1.42	157.5 ± 1.05
HIP extract (g/kg)	80.1 ± 2.63	49.9	0.0148	0.374	<0.01	80.5 ± 4.23	79.7 ± 3.24
Fillet out of live weight (%)	37.93 ± 0.267	36.86	0.00052	0.131	>0.05	38.24 ± 0.293	37.62 ± 0.445
Live weight (g)	2 044 ± 66.9					2 164 ± 96.9	1 923 ± 87.3

HIP = hexane/2-propanol
X = live weight of carp in g (1 172 ≤ X ≤ 3 196)
a, b = parameters of equation
r = correlation coefficients

[1] $n = 48$
[2] significance of regression coefficient b
[3] $n = 24$

of the same age increased significantly ($P < 0.05$) by 10.8 mg/kg with each gram of live weight. The value of HIP extract increased by 14.8 mg/kg ($P < 0.01$) while the content of crude protein decreased by 4.7 mg/kg ($P < 0.05$; Table 1). This means that meat of fish with a higher growth rate had a higher content of dry matter and fat and a lower content of protein. Corraze *et al.* (1993) reported similar data regarding trout.

Fatty acid content

The pattern of fatty acids and its dependence on the live weight of carp is presented in Table 2. Carp fillets contained much less n-3 PUFA and n-6 PUFA, substantially more MUFA, and the n-3 to n-6 PUFA ratio was much lower than the values published for carp by Kinsella *et al.* (1978), Sýkora and Valenta (1978), Kim and Lee (1986) and Vácha

Table 2. Fatty acid pattern and its dependence on live weight of carp

Fatty acids	Mean ± standard error of the mean [1]	$Y = a + bX$ [1]				Females[3]	Males[3]
		a	*b*	*r*	*P* [2]	mean ± standard error of the mean	
C14 : 0	1.40 ± 0.021	1.60	–0.000100	0.316	<0.05	1.37 ± 0.023	1.42 ± 0.035
C16 : 0	21.44 ± 0.113	21.94	–0.000246	0.145	>0.05	21.36 ± 0.170	21.51 ± 0.152
C16 : 1	10.73 ± 0.120	10.42	0.000152	0.085	>0.05	10.94 ± 0.164	10.52 ± 0.167
C18 : 0	5.86 ± 0.065	5.66	0.000100	0.102	>0.05	5.85 ± 0.076	5.88 ± 0.108
C18 : 1 n-9	45.97 ± 0.257	43.04	0.001432	0.372	<0.01	46.20 ± 0.310	45.73 ± 0.412
C18 : 2 n-6	6.91 ± 0.108	7.96	–0.000513	0.319	<0.05	6.74 ± 0.141	7.08 ± 0.158
C18 : 3 n-6	0.24 ± 0.007	0.31	–0.000033	0.333	<0.05	0.24 ± 0.010	0.24 ± 0.009
C18 : 3 n-3	1.63 ± 0.049	2.22	–0.000288	0.396	<0.01	1.61 ± 0.059	1.66 ± 0.079
C20 : 1 n-9	2.28 ± 0.047	1.78	0.000241	0.346	<0.05	2.23 ± 0.042	2.33 ± 0.083
C20 : 4 n-6	0.97 ± 0.023	1.24	–0.000132	0.379	<0.01	0.93 ± 0.030	1.01 ± 0.035
C20 : 5 n-3	1.05 ± 0.040	1.56	–0.000253	0.420	<0.01	1.03 ± 0.047	1.07 ± 0.066
C22 : 4 n-6	0.08 ± 0.003	0.10	–0.000011	0.254	>0.05	0.08 ± 0.004	0.09 ± 0.004
C22 : 5 n-3	0.36 ± 0.012	0.53	–0.000082	0.467	<0.001	0.35 ± 0.013	0.38 ± 0.019
C22 : 5 n-6	0.01 ± 0.001	0.01	0.000001	0.030	>0.05	0.01 ± 0.002	0.01 ± 0.002
C22 : 6 n-3	1.07 ± 0.039	1.62	–0.000269	0.457	<0.01	1.06 ± 0.052	1.08 ± 0.060
Σ SFA	28.69 ± 0.135	29.20	–0.000246	0.122	>0.05	28.58 ± 0.189	28.81 ± 0.194
Σ MUFA	58.97 ± 0.254	55.24	0.001826	0.481	<0.001	59.37± 0.321	58.58 ± 0.382
Σ PUFA	12.33 ± 0.230	15.56	–0.001579	0.459	<0.01	12.05 ± 0.283	12.61 ± 0.360
Σ (n-6)	8.21 ± 0.126	9.62	–0.000688	0.366	<0.05	8.00 ± 0.160	8.42 ± 0.188
Σ (n-3)	4.12 ± 0.131	5.94	–0.000891	0.455	<0.01	4.05 ± 0.155	4.19 ± 0.214
Σ (n-3)/Σ (n-6)	0.500 ± 0.0124	0.632	–0.0000644	0.347	<0.05	0.505 ± 0.0154	0.495 ± 0.0197

SFA = saturated fatty acids

MUFA = monounsaturated fatty acids

PUFA = polyunsaturated fatty acids

[1] $n = 48$

[2] significance of regression coefficient *b*

[3] $n = 24$

Y = % out of total determined fatty acids

X = live weight of carp in g ($1\,172 \leq X \leq 3\,196$)

a, *b* = parameters of equation

r = correlation coefficient

Table 3. Changes of nutrient content in meat after heat treatment

Component	Raw meat	Stewed meat	Difference[1)]	P
Dry matter (g/kg)	262.2	280.8	–18.60 ± 1.862	<0.001
Crude protein (N × 6) in DM (g/kg)	587.9	583.9	3.99 ± 4.577	>0.05
HIP extract in DM (g/kg)	304.1	312.8	–8.75 ± 5.508	>0.05

DM = dry matter
HIP = hexane/2-propanol
P = significance of mean difference
n = 24
[1)]mean difference ± standard error of mean difference

Table 4. Changes in fatty acid pattern in meat after heat treatment

Fatty acids[1)]	Raw meat	Stewed meat	Difference[2)]	P
C14 : 0	1.389	1.371	0.018 ± 0.0096	>0.05
C16 : 0	21.437	21.405	0.032 ± 0.0142	<0.05
C16 : 1	10.486	10.423	0.064 ± 0.0399	>0.05
C18 : 0	5.975	6.044	–0.069 ± 0.0351	>0.05
C18 : 1 n-9	46.210	46.286	–0.076 ± 0.0532	>0.05
C18 : 2 n-6	6.968	6.929	0.039 ± 0.0196	>0.05
C18 : 3 n-6	0.222	0.227	–0.004 ± 0.0041	>0.05
C18 : 3 n-3	1.574	1.552	0.022 ± 0.0133	>0.05
C20 : 1 n-9	2.325	2.387	–0.062 ± 0.0168	<0.01
C20 : 4 n-6	0.974	0.970	0.004 ± 0.0098	>0.05
C20 : 5 n-3	0.997	0.989	0.008 ± 0.0113	>0.05
C22 : 4 n-6	0.088	0.088	0.000 ± 0.0012	>0.05
C22 : 5 n-3	0.351	0.342	0.008 ± 0.0034	<0.05
C22 : 5 n-6	0.020	0.018	0.002 ± 0.0009	<0.05
C22 : 6 n-3	0.983	0.969	0.015 ± 0.0129	>0.05
Σ SFA	28.801	28.820	–0.019 ± 0.0236	>0.05
Σ MUFA	59.021	59.096	–0.075 ± 0.0351	<0.05
Σ PUFA	12.178	12.084	0.094 ± 0.0496	>0.05
Σ (n-6)	8.273	8.232	0.041 ± 0.0251	>0.05
Σ (n-3)	3.905	3.852	0.052 ± 0.0269	>0.05
Σ (n-3)/ Σ (n-6)	0.471	0.468	0.004 ± 0.0018	>0.05

SFA = saturated fatty acids
MUFA = monounsaturated fatty acids
PUFA = polyunsaturated fatty acids
P = significance of mean difference
[1)]% out of total determined fatty acids
[2)]mean difference ± standard error of mean difference
n = 24

and Tvrzická (1995). Also Viola *et al.* (1988) and Geri *et al.* (1995b) observed higher PUFA values as compared to our data. On the other hand, the n-3 to n-6 PUFA ratio found in the present experiment was similar to that reported by Geri *et al.* (1995b) in carp reared in water of natural temperature, and ranged within the limits observed for carp by Bieniarz *et al.* (2000).

The content of MUFA increased significantly ($P < 0.001$) with the increasing weight of fish of the same age in the present experiment while that of PUFA decreased ($P < 0.01$). The ratio of n-3 to n-6 PUFA decreased significantly ($P < 0.05$) with increasing growth rate. However, the decrease in n-3 PUFA was more pronounced ($P < 0.01$) than that of n-6 PUFA ($P < 0.05$). Intramuscular lipid content influenced the FA composition of muscles; with the increasing content of lipids, the percentage of MUFA increased ($P < 0.001$) while that of PUFA decreased ($P < 0.01$). Geri *et al.* (1995a) similarly observed a linear decrease in the content of C20 : 4 and C22 : 6 and an increase in the level of MUFA with increasing body weight of fish, however in a methodologically different experiment where fish of different age were evaluated.

Fish showing a higher growth rate, covering probably a greater part of food intake by wheat, accumulated more oleic acid in the meat ($P < 0.01$). Similar results were also reported by Farkas *et al.* (1978), Csengeri *et al.* (1978), Watanabe *et al.* (1981), Runge *et al.* (1987) and Schwarz *et al.* (1988). Probably desaturation of SFA synthetized from a starchy diet with low content of linoleic acid and α-linolenic acid led to an increase in the content of MUFA and a simultaneous decrease in PUFA content (Henderson, 1996).

Effect of heat treatment

Dry matter content of stewed meat was 1.9 per cent higher ($P < 0.001$) than that of raw meat (Table 3). The content of crude protein and HIP extract in dry matter of raw and stewed meat was not influenced by the heat treatment ($P > 0.05$).

Changes of the fatty acid pattern in meat due to the heat treatment are summarised in Table 4. Similarly to the results of Yamamoto and Imose (1989) and Tothmarkus and Sasskiss (1993), all these changes were very small, but contrary to the values published by Myers and Harris (1975), the percentage of C16 : 0, C22 : 5 n-3 and C22 : 5 n-6 in heat-treated fillets was significantly lower ($P < 0.05$) in comparison with the raw meat in the present experiment. On the other hand, the percentage of C20 : 1 n-9 increased significantly ($P < 0.01$) due to the heat treatment (Table 4). Despite of the above-mentioned significant effect of stewing on the percentage of some fatty acids in the present experiment, the absolute differences in the fatty acid pattern in natural and cooked meat were substantially lower in comparison with data of Quaglia *et al.* (1974). The ratio of n-3/n-6 PUFA in the meat was not changed by the heat treatment in the present experiment ($P > 0.05$).

CONCLUSIONS

Common carp of the same age with body weight ranging from about 1 200 to 3 200 g at the moment of autumn harvest did not show sexual dimorphism in the fatty acid pattern of meat. Higher content of dry matter and fat and lower content of protein was observed in meat of fish with higher growth rate. The proportion of SFA in the total content of FA was stable, but the percentage of MUFA was significantly higher, while that of PUFA was significantly lower in fish growing with higher intensity. The ratio of n-3 to n-6 PUFA in fillets of heavier fish of the same age was less favourable (i.e. lower) for consumers. Stewing changed the fatty acid pattern of fish fillets only very slightly.

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Received: 02–10–02
Accepted after corrections: 03–01–10

ABSTRAKT

Vliv pohlaví, intenzity růstu a tepelné úpravy na zastoupení mastných kyselin v mase kapra *(Cyprinus carpio)*

Z kaprů chovaných v pokusném rybníce a přikrmovaných pšenicí bylo ve třetím roce života vybráno 48 jedinců. Živá hmotnost ryb se pohybovala v rozpětí 1 172 až 3 196 g. Kapři byli nafiletováni a ve filé bez kůže byl stanoven

obsah mastných kyselin metodou plynové chromatografie po extrakci celkových lipidů směsí hexan/2-propanol. Pohlavní dimorfismus v chemickém složení masa kaprů z podzimního výlovu byl neprůkazný ($P > 0,05$). Se zvyšováním rychlosti růstu se obsah sušiny a tuku ve svalovině lineárně zvyšoval, zatímco obsah bílkovin klesal ($P < 0,05$). Procentický podíl nasycených mastných kyselin se prakticky neměnil ($P > 0,05$), zastoupení mononenasycených mastných kyselin se zvyšovalo ($P < 0,001$) a podíl polynenasycených mastných kyselin (PUFA) klesal ($P < 0,01$). Pokles n-3 PUFA byl rychlejší než pokles n-6 PUFA. Poměr n-3/n-6 PUFA byl u větších ryb stejného věku méně výhodný pro spotřebitele. Vliv tepelné úpravy dušením na podíl mastných kyselin byl velmi malý. Pokles C16 : 0, C22 : 5 n-3 a C22 : 5 n-6 však byl přesto průkazný ($P < 0,05$) a vzestup C20 : 1 dokonce vysoce průkazný ($P < 0,01$).

Klíčová slova: *Cyprinus carpio*; mastné kyseliny; růst; tepelná úprava masa; pohlavní dimorfismus

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Isolation of the psychrotolerant species *Bacillus weihenstephanensis* from raw cow's milk

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ABSTRACT: Fifty-six mesophilic and psychrotolerant *Bacillus cereus* strains were isolated during a monitoring of *Bacillus* spp. in bulk milk samples from Moravian and Bohemian farms, Czech Republic. Thirty-four mesophilic strains grew at 40–43°C and did not grow at 4–7°C. Twenty of the 22 psychrotolerant strains were able to grow between 10 and 40°C. They did not grow at 4 and 43°C. Only two cold-tolerant strains that were classified as *Bacillus weihenstephanensis* were able to grow at 4–7°C. Two PCR assays (*cspA*-PCR and 16S rDNA-PCR) and ribotyping confirmed their correct identification as *B. weihenstephanensis*. These strains are deposited in the Czech Collection of Microorganisms (CCM) as *B. weihenstephanensis* CCM 4965 and CCM 4966.

Keywords: *Bacillus cereus*; *Bacillus weihenstephanensis*; growth data; identification; 16S rDNA-PCR; *cspA*-PCR; ribotyping; raw milk

Spores of the genus *Bacillus* are commonly found in raw milk. They are important contaminants penetrating from milk to pasteurized milk products. Besides predominant mesophilic species, e.g. *B. licheniformis, B. subtilis* and *B. pumilus,* dominant psychrotrophic isolates are represented by *B. cereus* strains (Sutherland and Murdoch, 1994; Crielly *et al.,* 1994; Te Giffel *et al.,* 1997; Lukášová *et al.,* 2001; Stenfors and Granum, 2001). Most incidents of food poisoning and food-borne illnesses attributed to *Bacillus* species are associated with *Bacillus cereus*, which limits the keeping quality of pasteurized milk. The spore-forming, facultative anaerobic *B. cereus* produces emetic and diarrhoeal type of enterotoxins and is ubiquitously distributed (Granum and Lund, 1997; Rowan and Anderson, 1998; Borge *et al.,* 2001) and therefore new rapid methods for *B. cereus* determination in foods, including milk and dairy products are still developed. A combination of phenotypic properties, serotyping, toxin production, plasmid profiling and PCR methods is used for *B. cereus* detection (Logan and Berkeley, 1984; Shinagawa, 1993; Granum and Lund, 1997; Te Giffel *et al.,* 1997; Nilsson *et al.,* 1998; Pruss *et al.,* 1999; Pirttijärvi *et al.,* 1999; Borge *et al.,* 2001; Torkar and Možina, 2000, 2001).

Even if *B. cereus* is not traditionally considered a psychrotrophic species (Stenfors and Granum, 2001), during the last years there appeared many reports (Meer *et al.,* 1991; Sutherland and Murdoch, 1994; Larsen and Jørgensen, 1999; Mayr *et al.,* 1999; von Stetten *et al.,* 1999; Pirttijärvi *et al.,* 1999) on the occurrence of psychrotolerant strains belonging to the "*Bacillus cereus* group". The new species, *Bacillus weihenstephanensis* has been proposed to accommodate psychrotolerant *Bacillus cereus* strains (Lechner *et al.,* 1998). Isolates of this new species grow at 4–7°C but not at 43°C and can be identified rapidly using 16S rDNA and *cspA* targeted PCRs. Daffonchio *et al.* (2000), who studied the genetic relationships within six species of the "*B. cereus* group", stated that *B. weihenstephanensis* was closely related to *B. mycoides* and

*Supported in part by Ministry of Agriculture of the Czech Republic (Grant No. EP9058).

Table 1. Growth data on 56 mesophilic and psychrotolerant strains of "*Bacillus cereus* group"

	Number of strains	Growth at			
		4 and 7°C	10°C	40°C	43°C
Group I (mesophilic strains)	34	–	–	+	+
Group II (psychrotolerant strains)	20	–	+	– or single colonies	–
B. weihenstephanensis	2	+	+	–	–

B. paramycoides by phenotype and genotype while *B. anthracis, B. cereus* and *B. thuringiensis* clustered in a separate group. So far *B. weihenstephanensis* strains have been isolated from milk and meat products (Lechner *et al.*, 1998; Mayr *et al.*, 1999; Stenfors and Granum, 2001) and alpine soil (von Stetten *et al.*, 1999).

Frequencies of psychrotrophic, thermoresistant and spore-forming bacteria are monitored as a complementary trait of hygienic quality testing of raw cow's milk (Czech Standard CSN 57 0529). Fifty-six mesophilic and psychrotolerant *B. cereus* strains were identified during a monitoring of *Bacillus* strains in bulk milk samples from Moravia and Bohemia, Czech Republic. All strains were described using morphological, physiological and biochemical reactions (Vyletělová *et al.*, 2001). The tested mesophilic and psychrotolerant strains were lecithinase, phosphatase, gelatinase, esterase and haemolysis positive (except one lecithinase negative strain) and produced acetoin, hydrolysed esculin and starch and grew in anaerobic conditions (Vyletělová *et al.*, 2001). To confirm psychrotolerance, the strains were cultivated both on solid and in liquid Plate Count Medium and incubated at 4, 7 and 10°C for up to two weeks (Table 1). The *B. cereus* mesophilic group (34 isolates) grew well at 40–43°C and did not grow at 4–7°C. Twenty of the 22 psychrotolerant strains were able to grow between 10 and 40°C and did not grow at 4°C and 43°C. These results confirmed the occurrence of intermediate forms between *B. cereus* and *B. weihenstephanensis* species as reported by Pruss *et al.* (1999) and Stenfors and Granum (2001). Only two of the cold-tolerant strains (B22 and B23) that were classified provisionally as *B. weihenstephanensis* (Vyletělová *et al.*, 2001) were able to grow at 4–7°C.

To clarify and verify the taxonomic position of the two isolated *B. weihenstephanensis* strains *cspA*-PCR, 16S rDNA-PCR and ribotyping were used.

The polymerase chain reactions were performed as described by Stenfors and Granum (2001). Two methods for discriminating mesophilic strains from psychrotolerant ones were developed by Francis *et al.* (1998) and von Stetten *et al.* (1998). One is based on the amplification of a segment of the cold shock protein A gene (*cspA*), where a specific signature sequence, making amplification possible, is present only in psychrotolerant strains. The other method takes advantage of sequence differences between the 16S ribosomal genes in the two types of strains. From both the presumptive *B. weihenstephanensis* strains in this study, the correct psychrotolerant fragments were amplified by these methods, confirming the species identity.

Ribotyping with *Eco*RI restriction enzyme and 16S+23S rRNA genes complementary probe was done as described by Švec *et al.* (2001). In full agreement with both PCR based methods this method confirmed that both strains represent *B. weihenstephanensis* species (Figure 1). Both *B. weihenstephanensis* strains (CCM 4965 and CCM 4966) clustered together with *B. weihenstephanensis* CCM 4872^T type strain. Moreover, this cluster was clearly separated from *B. cereus* CCM 2010^T type strain as well as from representative *B. cereus* strains chosen from mesophilic and psychrotolerant groups described in Table 1. Although ribotyping is not considered to be a species-specific method, our results indicate that it could be used for differentiation of *B. weihenstephanensis* and *B. cereus* species.

Even if Christiansson *et al.* (1989) believed that psychrotolerant strains of *B. cereus* from dairy products are hardly able to produce enterotoxins, it is conceivable that besides mesophilic *B. cereus* strains also psychrotolerant strains including *B. weihenstephanensis* may play a role in spoilage and poisoning of low temperature stored and minimally processed foods and from this aspect it is necessary to monitor *B. weihenstephanensis* and other psychrotolerant *B. cereus* during food microbial quality testing.

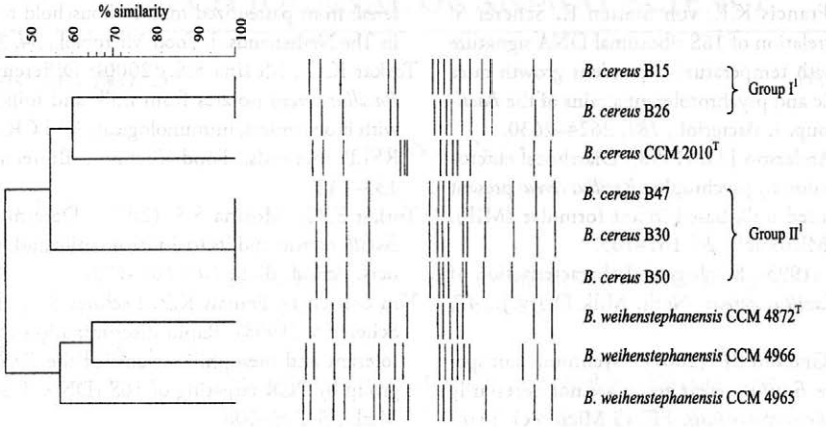

Figure 1. Ribotype patterns obtained with *Eco*RI restriction enzyme and a probe complementary to 16S and 23S rRNA genes

[1]see Table 1 for characterization of Groups I and II

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Received: 02–10–15
Accepted after corrections: 02–12–07

ABSTRAKT

Izolace psychrotolerantního druhu *Bacillus weihenstephanensis* ze syrového kravského mléka

Celkem 56 mezofilních a psychrotolerantních kmenů *Bacillus cereus* bylo izolováno při monitorování výskytu druhů rodu *Bacillus* v bazénových vzorcích mléka v oblasti Moravy a Čech. 34 mezofilních kmenů rostlo při teplotě 40–43 °C a nerostlo při 4–7 °C. Dvacet z 22 psychrotolerantních kmenů rostlo v rozmezí 10–40 °C, ale nerostlo při teplotě 4 °C ani 43 °C. Pouze dva psychrotolerantní kmeny *Bacillus weihenstephanensis* rostly při teplotě 4–7 °C. Jejich druhová identifikace byla potvrzena i dvěma metodami PCR (*cspA*-PCR a 16S rDNA-PCR) a ribotypizací. Kmeny jsou uloženy v České sbírce mikroorganismů (CCM) jako *B. weihenstephanensis* CCM 4965 a CCM 4966.

Klíčová slova: *Bacillus cereus*; *Bacillus weihenstephanensis*; růstová data; identifikace; 16S rDNA-PCR; *cspA*-PCR; ribotypizace; syrové mléko

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An international scientific journal
published under the auspices of the Czech Academy of Agricultural Sciences
and financed by the Ministry of Agriculture of the Czech Republic

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